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Antimicrobial Evaluation of Aqueous Leaf Extract of Aerva Lanata: A Phytopharmacological Study

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Abstract: Medicinal plants have long been recognized as valuable sources of therapeutic agents, particularly in the management of microbial infections. The present study aimed to evaluate the antimicrobial potential of the aqueous leaf extract of Aerva lanata, a traditionally used medicinal plant belonging to the family Amaranthaceae. The plant leaves were collected, authenticated, shade-dried, and subjected to aqueous extraction using the Soxhlet method. Preliminary phytochemical screening revealed the presence of bioactive constituents such as carbohydrates, tannins, proteins, and resins. The antimicrobial activity of the extract was assessed against selected Gram-negative bacterial strains, including Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, and Fusobacterium nucleatum, as well as fungal strains such as Candida albicans and Aspergillus niger, using the agar well diffusion method. The results demonstrated that the aqueous extract exhibited moderate to significant antimicrobial activity, with zones of inhibition varying depending on concentration and microbial strain. The highest activity was observed at 500 µg/mL concentration, showing comparable effects to standard drugs such as gentamicin. The findings suggest that Aerva lanata possesses promising antimicrobial properties, which may be attributed to its phytochemical constituents. This study supports the traditional use of the plant and highlights its potential as a natural source for the development of antimicrobial agents.

Keywords: Aerva lanata, Antimicrobial activity, Aqueous leaf extract, Phytochemical screening, Agar well diffusion method, Medicinal plants

1. INTRODUCTION

Medicinal plants have been an integral part of traditional healthcare systems for centuries and continue to play a vital role in modern therapeutics. A significant proportion of the global population, particularly in developing countries, relies on plant-based medicines for primary healthcare due to their accessibility, affordability, and perceived safety. These plants contain diverse bioactive compounds that contribute to their pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory effects [1]. In recent decades, the emergence of antimicrobial resistance has become a major global health challenge. The widespread and often indiscriminate use of antibiotics has led to the development of resistant strains of microorganisms, resulting in reduced efficacy of conventional drugs and increased treatment failures [2]. Pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* have shown increasing resistance to commonly used antibiotics, posing serious threats to public health [3]. Consequently, there is an urgent need to identify novel and effective antimicrobial agents, particularly from natural sources.

Plants are considered a rich reservoir of antimicrobial compounds, including alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds. These phytochemicals exert antimicrobial effects through

various mechanisms such as disruption of microbial cell membranes, inhibition of enzyme activity, and interference with nucleic acid synthesis [4]. The exploration of plant-derived antimicrobial agents has gained considerable attention as an alternative strategy to combat drug-resistant pathogens.

Aerva lanata (L.) Juss. ex Schultes, belonging to the family Amaranthaceae, is a widely distributed medicinal herb found throughout India. It is commonly known as "bui" and has been extensively used in traditional medicine for the treatment of various ailments such as kidney stones, urinary disorders, inflammation, cough, and microbial infections [5]. The plant possesses a wide range of pharmacological activities, including diuretic, anti-inflammatory, antioxidant, antidiabetic, antimicrobial, and hepatoprotective effects [6].

Phytochemical studies of *Aerva lanata* have revealed the presence of several bioactive constituents such as alkaloids, flavonoids, glycosides, tannins, saponins, and phenolic compounds. These constituents are believed to be responsible for its therapeutic efficacy and biological activities [7]. Previous research has demonstrated that extracts of *Aerva lanata* exhibit antimicrobial activity against a variety of bacterial and fungal pathogens, indicating its potential as a natural antimicrobial agent [8].

Despite the traditional use and reported pharmacological properties of *Aerva lanata*, systematic evaluation of its aqueous leaf extract for antimicrobial activity remains limited. Therefore, scientific validation of its antimicrobial potential is essential to support its use in herbal medicine and to explore its possible application in drug development.

The present study aims to evaluate the antimicrobial activity of the aqueous leaf extract of *Aerva lanata* using standard microbiological techniques. Additionally, preliminary phytochemical screening was performed to identify the active constituents responsible for its antimicrobial effects.

1.1. Taxonomical Classification

Kingdom: Plantae (Plants)

Sub-kingdom: Tracheobionta (Vascular plants)

Division: Magnoliophyta (Angiospermes, fl owering plants)

Class: Magnoliopsida (Dicotylédones)

Subclass: Caryophyllidae

Order: Caryophyllales

Family: Amaranthaceae

Genus: Aerva

Species: *Aerva lanata* (L.) A. L. Juss. ex Schultes

Used Part: Leaf

Common name

Ayurvedic: Paashaanabheda, Gorakshaganjaa, Aadaanpaaki, Shatkabhedi

Bengali: Chaya

Rajasthani: Bhui

Sindhi: Bhui, Jari

Punjabi: Bui-kaltan

Hindi: Gorkhabundi, Kapurijadi

Aim:

To Antimicrobial Evaluation of Aqueous Leaf Extract of *Aerva lanata*: A Phytopharmacological Study

Objective:

- Because of their therapeutic qualities, the water-based extract from Aerva lanata leaves holds significant importance.
- It is beneficial for improving both memory and the ability to remember things. Furthermore, it is quite helpful in relieving headaches. It is useful in addressing eye conditions such as conjunctivitis, which is commonly called pinkeye. It demonstrates potency in mitigating virtually every kind of bleeding.
- The procedure incorporated securing and confirming the authenticity of, as well as working with, the materials taken from Aerva lanata leaves.
- The technique encompasses refining, dehydrating, and milling the water-based extract from Aerva lanata leaves into a powdered form.
- A Microbiological Assessment of the Water-Based Extract from Aerva lanata Leaves: A Study in Phytopharmacology.
- The study focuses on examining the investigations carried out concerning the Phytopharmacological and Antimicrobial qualities found in the Water-Based Leaf Extract derived from Aerva lanata.

2. MATHERIALS AND METHODS

2.1. Collection and Authentication of Plant Material

Fresh leaves of *Aerva lanata* were collected and plant material was identified and authenticated by Dr. P. Shivakumar Singh, Assistant Professor @ department of Botany at Palamuru University (voucher specimen number HPU: 510/2026). The collected leaves were washed, shade-dried, and powdered using a mechanical grinder. The powdered material was sieved (No. 40) and stored in airtight containers for further use.

2.2. Preparation of Aqueous Extract

The powdered leaves were subjected to aqueous extraction using the Soxhlet apparatus. Approximately 14 g of dried plant material was placed in a cellulose thimble and extracted with distilled water for about 4–72 hours. The solvent was heated, condensed, and continuously recycled through the sample. The extract obtained was concentrated by distillation and dried using a vacuum evaporator to yield a solid mass, which was stored for further analysis.

2.3. Phytochemical Screening

Preliminary qualitative phytochemical analysis of the aqueous extract was carried out using standard methods to detect the presence of various bioactive compounds. Tests were performed for carbohydrates, tannins, alkaloids, flavonoids, glycosides, steroids, phenols, saponins, proteins, resins, and other constituents based on color reactions and precipitation methods.

2.4. Test Microorganisms

The antimicrobial activity was evaluated against selected microorganisms including Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Fusobacterium nucleatum*, and fungal strains such as *Candida albicans* and *Aspergillus niger*. The cultures were obtained from MTCC, Chandigarh, India, and maintained on appropriate media.

3. QUALITATIVE ANALYSIS OF PHYTOCHEMICALS

The aqueous extracts of *Aerva lanata* were subjected to qualitative tests for the detection of various plant constituents (Kokate CK.,), (Krishnaswamy NR.,), (Surekha yarnalkar.), (Khandelwal KR.,), (Rajgopal G.,).

- Detection of Carboxylic acid

To 1ml plant extract, 2ml of sodium bicarbonate solution is added. Colour changes occur indicates the presence of carboxylic acid.

- Detection of Tannins

To 2ml of plant extract, 2-3ml of 10% HCL is added and boiled for 5-6 min. Formation of red colour indicates the presence of tannins.

- Detection of Steroids

To 0.5 ml extract, 5ml of chloroform is added and equal amount of conc. H2so4 was added. In the upper layer formation of red colour and in the lower layer, yellow with green colour is formation indicates the presence of steroids.

- Detection of Flavanoids

To 0.5 ml extract, 4ml of 1% ammonia was added and to this 1ml of conc. H2so4was added. The formation of yellow colour indicates the presence of flavonoids.

- Detection of Glycosides
- Born- Trageru's Test

Taken 2ml of hydrolysate, 3ml of chloroform was added, shaken vigorously, then the chloroform layer gets separated. Then 10% ammonia solution was added. The formation of pink colour indicates the presence of glycosides.

- Detection of Proteins (Bradford Method)

To 500µl of plant extract, 5ml of the Bradford reagent was added, incubated at dark for 10 to 15 min. taken the OD at 575nm.

- Detection of Phenol (Ferric Chloride Test)

To 50 mg of extract, 5ml of distilled water was added and a few drops of 5% ferric chloride solution were added. The formation of dark green colour indicates the presence of phenol.

- Saponin Test

To 50 mg of plant extract, 20 ml of distilled water was added and shaken vigorously for 15 min, formation of 2 cm layer of foam indicates the presence of saponins.

- Test for Alkaloids - Mayer's test

To a few ml of plant sample extract, two drops of Mayer's reagent are added along the sides of the test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

- Saponification test

To 1 or 2ml of 10 N sodium hydroxide, 2ml of extract is added and boiled for 2 minutes formation of soap or fat indicates the positive test for saponification.

- Gum Test

The 100 mg of plant extract was dissolved in 2 ml of distilled water.2ml of absolute alcohol with constant stirring. White colour cloudy precipitate indicates gums & mucilage's.

- Detection of flavanoglycoside

The 50 mg of plant extract was dissolved in 5ml ethanol. Added a few drops of magnesium sulfate & few drops of conc.HCL. The formation of pink colour indicates the presence of flavanoglycoside.

- Detection of Carbohydrates

To 0.5ml of extract, 0.5 ml of Benedict reagent was added ad boiled for 2 min. Color changes and precipitate are formed. It indicates the presence of carbohydrate.

- Detection of resins

To 0.5 ml of plant extract, 3 ml of CuSO4 solution is added. Shaken for about 1-2 min, formation of green colour precipitate indicates the presence of resins.

- Biuret test

To 2ml of extract, 1 drop of 2% CuSO4 solution. Add 1 ml of 95 % ethanol add 2 to 3 sodium hydroxide pellets. Formation of pink colour indicates the test positive.

3.1. Antibacterial Activity (Agar Well Diffusion Method)

The antibacterial activity of the aqueous extract was evaluated using the agar well diffusion method. Nutrient agar medium was prepared, sterilized, and poured into sterile petri plates. The plates were inoculated with 24-hour-old bacterial cultures adjusted to 0.5 McFarland standard.

Wells were created in the agar plates, and different concentrations of the extract (50, 100, 250, and 500 µg/mL) were introduced into the wells. The plates were incubated at 37°C for 24 hours. Gentamicin was used as the standard antibiotic. The antibacterial activity was determined by measuring the diameter of the zone of inhibition (in mm).

3.2. Antifungal Activity (Agar Well Diffusion Method)

Antifungal activity was assessed using the agar well diffusion method on potato dextrose agar medium. The fungal strains were inoculated into the medium, and wells were filled with different concentrations of the extract (50, 100, 250, and 500 µg/mL). The plates were incubated at 28°C for 72 hours. Amphotericin B was used as the standard antifungal agent. The antifungal activity was evaluated by measuring the zone of inhibition around the wells.

3.3. Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Data analysis was carried out using GraphPad Prism software (version 6.0), and the results were interpreted statistically.

4. RESULTS AND DISCUSSIONS

The leaves of *Aerva lanata* was selected for the detailed study to evaluate its phytochemical and biological properties. Literature survey revealed so for no work has been done on this fern claiming maximum therapeutic uses. So we felt worthwhile to validate scientifically, the folk claim for its therapeutic activity. We have also taken its detailed preliminary phytochemical investigations to prove its appropriate identification and rationalize its use as drug of therapeutic importance.

5. QUALITATIVE PHYTOCHEMICAL ANALYSIS

In the phytochemical studies, aqueous extracts of leaves of *Aerva lanata* showed the presence various phytoconstituents. Preliminary phytochemical screening of *Aerva lanata* revealed the presence of bioactive compounds such as glycosides, alkaloids, tannins, flavonoids, phenols and saponins in aqueous extract and fixed oil,gums and mucilage in petroleum ether extract. While the aqueous extract showed the presence of glycosides, alkaloids, flavonoids, steroids and phenols. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. The results are given in Table-1.

Table 1. *Data showing the preliminary phytochemical screening of the leaves extracts of Aerva lanata*

S. No	Phytoconstituents	*Aerva lanata* Aqueous Extract (D.H_2O)
1.	Carbohydrate	Present
2.	Glycosides	Present
3.	Alkaloids	Absent
4.	Steroids	Absent
5.	Tannins	Present
6.	Flavonoids	Present
7.	Phenols	Absent
8.	Saponins	Present
9.	Saponification	Absent
10.	Flavanoglycosides	Absent
11.	Gums	Absent
12.	Resins	Present
13.	Carboxylic acid	Absent
14.	Protein	Present
15.	Biuret	Absent

4.1. Antimicrobial Activity

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. In the present work, the extracts obtained *Aerva lanata* show strong activity against most of the tested bacterial and fungal strains. The results were compared with standard antibiotic drugs.

Table 2. *Antibacterial study of aqueous extract of Aerva lanata*

S. No	Name of the test organism	Name of the test sample	Zone of inhibition (mm) Mean±SD				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC(Gentamicin)
1.	*Pseudomonas aeruginosa*	AQEAL	13.75±1.06	12.3±0.42	0	0	15.4±0.56
2.	*Proteus mirabilis*		11.35±0.49	10.25±0.35	5.15±0.21	0	16.5±0.707
3.	*E.coli*		14.5±0.70	12.4±0.56	10.3±0.42	0	17.5±0.707
4.	*Fusobacterium nucleatum*		16±1.41	11.4±0.56	10.3±42	9.25±0.35	16.75±1.06
5.	*Klebsiella pneumoniae*		15.75±1.06	12.4±0.56	0	0	17.25±0.35

Table 3. *Antifungal study of aqueous extract of Aerva lanata*

S. NO	Name of the test organism	Name of the test sample	Zone of inhibition (mm)SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC (Amphotericin B)
1.	*Aspergillus niger*	AQEAL	13.5±0.707	7.25±0.35	0	0	19.75±1.06
2.	*Candida albicans*		12.75±1.06	11.35±0.49	6.2±0.28	5.1±0.141	15.5±0.707

4.2. Anti-Bacterial Activity

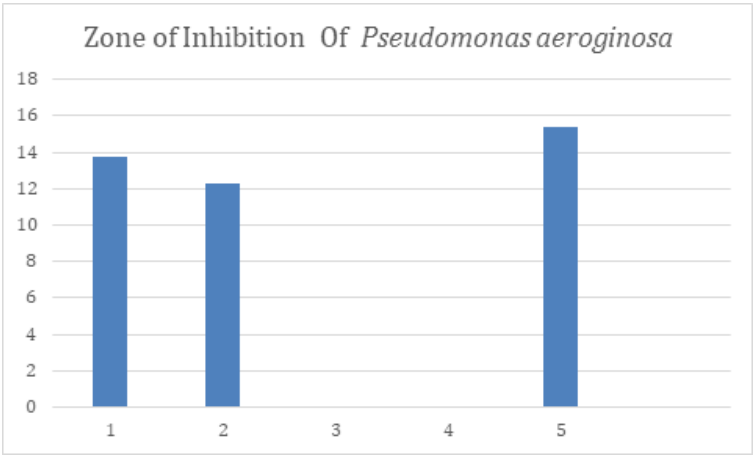

Fig- 1. *Antibacterial activity of aqueous extract of Aerva lanata against Pseudomonas aeroginosa*

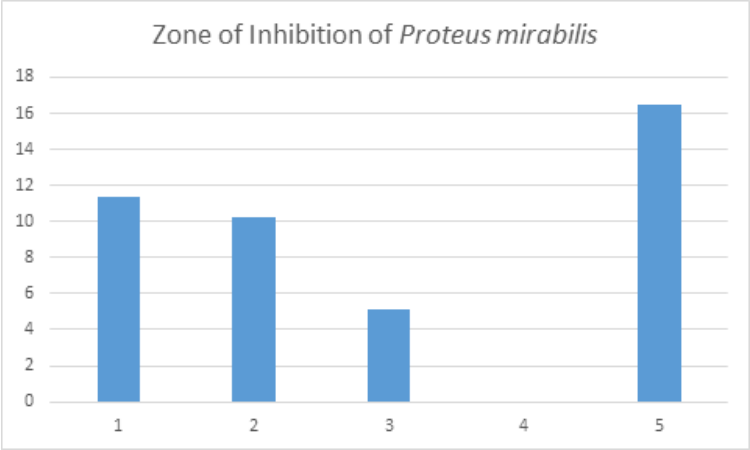

Fig- 2. *Antibacterial activity of aqueous extract of Aerva lanata against Proteus mirabilis*

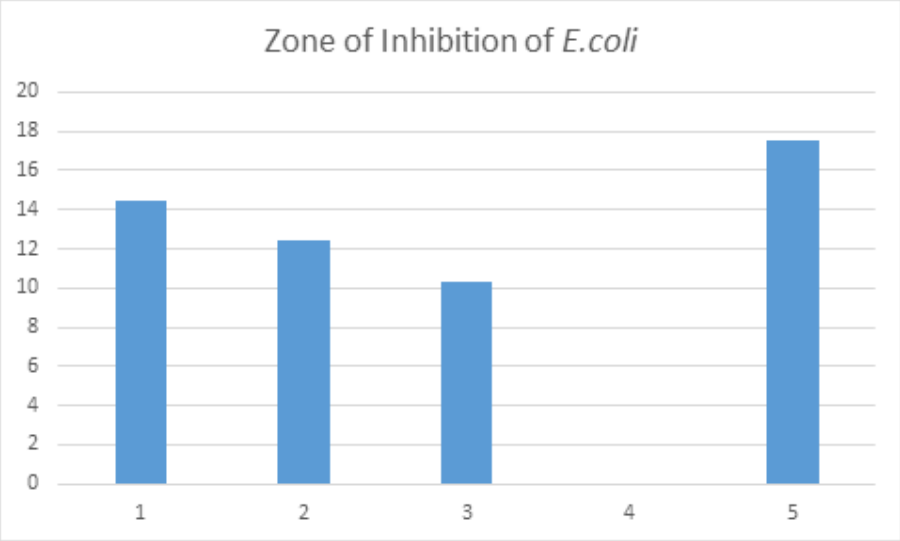

Fig- 3. *Antibacterial activity of aqueous extract of Aerva lanata against Escherichia coli*

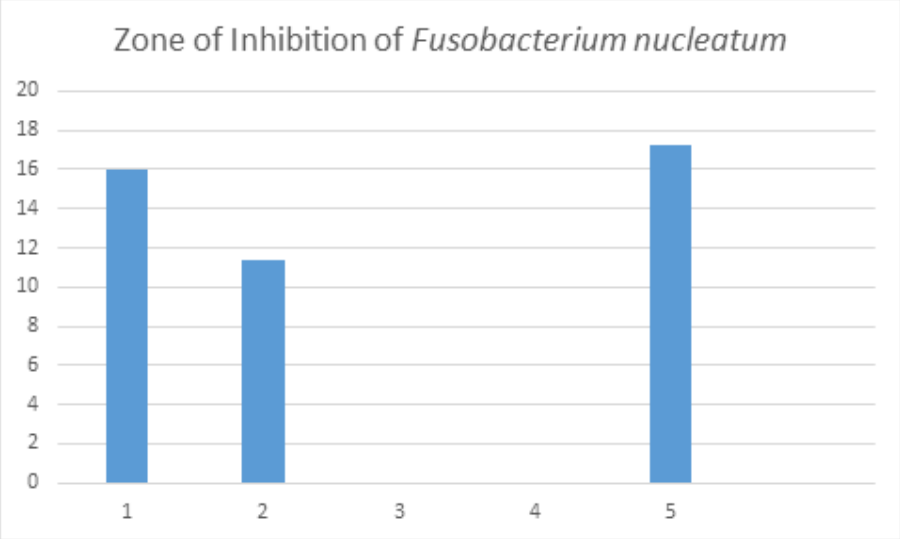

Fig- 4. *Antibacterial activity of aqueous extract of Aerva lanata against Fusobacterium nucleatum*

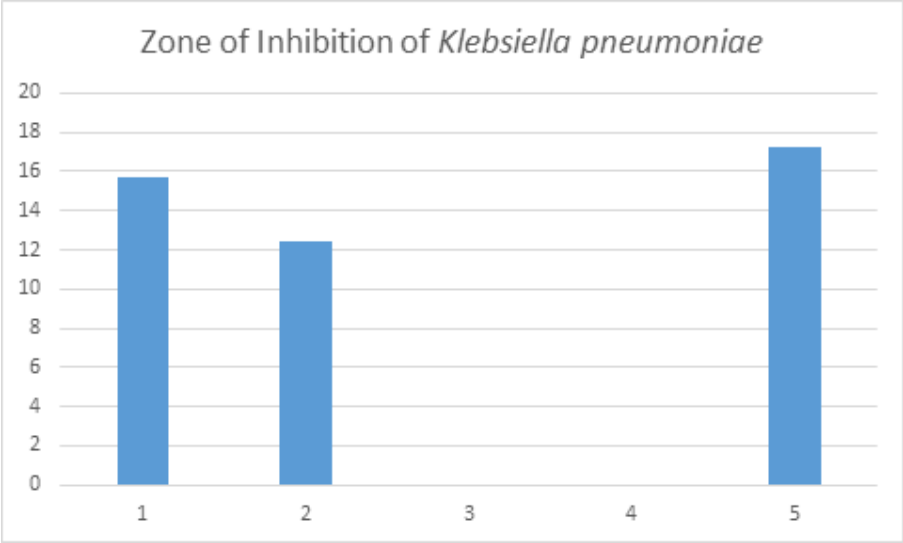

Fig-5. *Antibacterial activity of aqueous extract of Aerva lanata against Klebsiella pneumonia*

4.3. Antifungal Activity

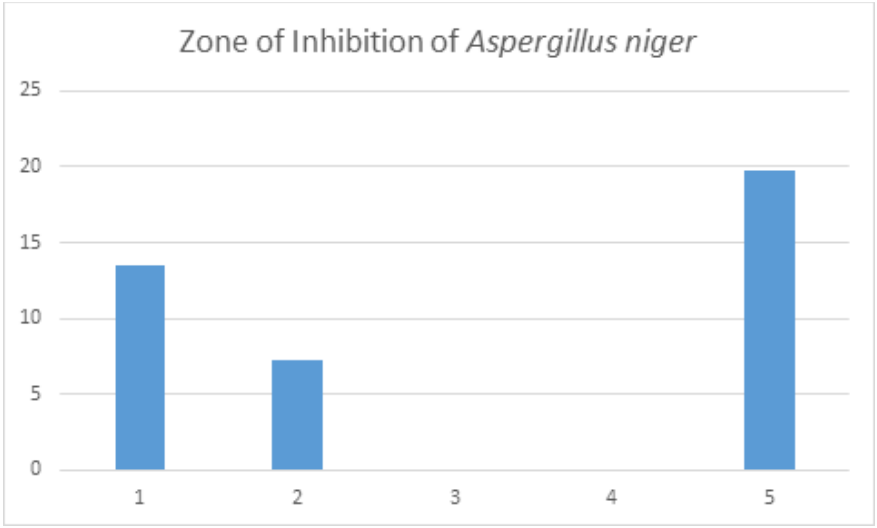

Fig-6. *Antifungal activity of aqueous extract of Aerva lanata against Aspergilus niger*

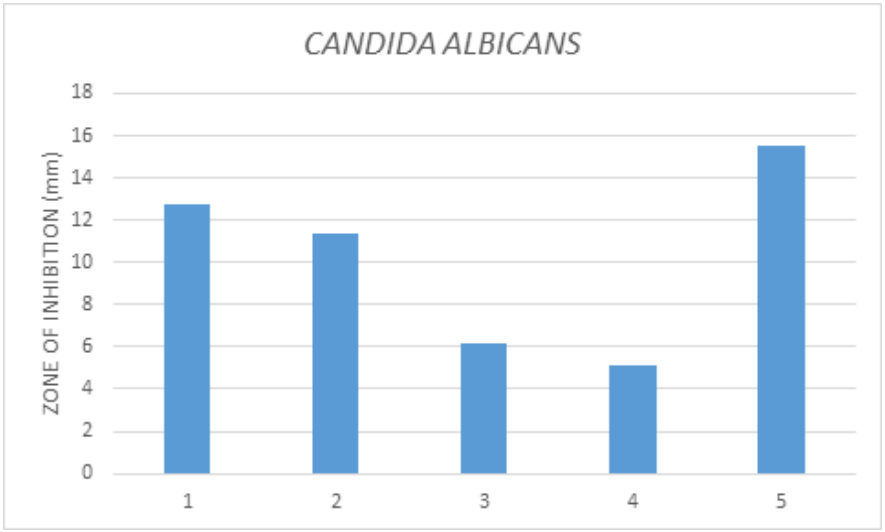

Fig-7. *Antifungal activity of aqueous extract of Aerva lanata against Candida albicans*

In the present study, the antimicrobial activity of leaves of *Aerva lanata* were performed by using agar diffusion method. The antimicrobial activity of the extracts was studied in different concentrations (50,100,250 and 500 µg/ml) against five pathogenic bacterial strains (Escherichia coli, Klebsiella pneumonia, proteus mirabilis, Fusobacterium nucleatum, and pseudomonas aeruginosa) and two fungal strains (Aspergillus niger &Candida albicans). These strains have been selected for the basis of its application purpose of further formulation study.

The antibacterial and antifungal properties of extracts were identified. The results indicated that, the extracts obtained from the plant showed inhibition of growth against tested microorganisms. Successful prediction of extracted compounds from plant material largely dependent on the type of solvents used in the extraction procedure. The aqueous solvent for extracting antimicrobial substance, aqueous extract of *Aerva lanata* reported to be more effective against fungal and bacterial species showing highest inhibition such as 13.5±0.707mm against fungi *A.niger* and 12.75±1.06mm against *C.albicans* and 16±1.41mm against bacteria *Fusobacterium nucleatum,* 15.75±1.06mm against *Klebsiella pneumonia,* 14.5±0.70 against *E.coli,* 13.75±1.06 against *Pseudomonas aeruginosa,* 11.35±0.49 against *Proteus mirabilis*.

The overall evaluation studies showed that the aqueous extract of *Aerva lanata* showed highest degree of antibacterial and antifungal activity.

The antimicrobial effect of ethanol extracts against these organisms may be due to the ability of the ethanol to extract some of the active properties of these plants glycosides, alkaloids, tannins, flavonoids, saponins and other secondary metabolites which are reported to be antimicrobial (Cowan MM., 1999),(Okwu DE., 2006). The results show that the aqueous extract of Aerva lanata was found to be more effective against all the microbes Tested Compared To Other Extracts.

6. SUMMARY AND CONCLUSION

- The term medicinal plants include plants have a medicinal activity. Medicinal plants used in drug development and synthesis, because of its rich resource of ingredients. There are about half million plants around the world and they have medicinal properties and it could be decisive in the treatment of future or present studies. In developed countries, plant drug constituent as much as 25% of the total drugs, while in fast developing countries, the contribution is as much as 80%. The economic importance of medicinal plants is much more to countries such as India than to the rest of the world. Only a relatively small fraction of the world's green pharmacy has been investigated for possible pharmacological and therapeutic properties. This holds the promise of investigation important phytoconstituents from plant origin.
- The leaves of *Aerva lanata* was selected for the detailed study to evaluate its phytochemical and biological properties. Literature survey revealed so for no work has been done on this fern claiming maximum therapeutic uses. So, we felt worthwhile to validate scientifically, the folk claim for its therapeutic activity. We have also taken its detailed preliminary phytochemical investigations to prove its appropriate identification and rationalize its use as drug of therapeutic importance.
- The plant materials were collected from the surrounding areas of Erode district and authenticated. The leaves were powdered and extracted with aquoues solvent using Soxhlet apparatus separately. The extract was concentrated to dryness under reduced pressure and controlled temperature. Qualitative phytochemical screening was carried out to identify the phytoconstituents.
- Preliminary phytochemical screening of *Aerva lanata* revealed the presence of bioactive compounds such as resins, carbohydrates, saponification and protein in aqueous extract.
- The antimicrobial activity of leaves of *Aerva lanata* was performed by using agar well diffusion method. The antibacterial and antifungal activities of the extract increased linearly with increase in concentration of extracts (µg/ml). As compared with standard drugs, the results revealed that in the extracts for bacterial activity, Streptomyces fulvissimus and Basillus subtilis were more sensitive as compared with Pseudomonas aeruginosa and for fungal activity Aspergillus niger shows good result as compare with Candida albicans . The growth inhibition zone measured ranged from 11-16mm for all the sensitive bacteria, and ranged from 10-15mm for fungal strains.The aqueous extract of *Barleriaprionitis* showed greater antimicrobial activities than other extracts

- The results suggested that the *Aerva lanata* had significant antibacterial activity against both gram (+ve) and gram (–ve) organism as well as antifungal activity. The antimicrobial potency of these plant extracts is due to the presence of alkaloids, tannins, flavonoids and saponins. It is interesting to note that even crude extract of these plants showed prominent activity against various pathogenic bacteria (Patil AS., 1993). The variation of the susceptibility of the tested microorganisms could be attributed to their intrinsic properties that are related to the permeability of their cell surface to the extracts.

7. CONCLUSION

In the present study, the herbal extract of Aerva lanata leaves examined for antimicrobial activity. The aqueous extract of Aerva lanata was found to be active on most of the clinically isolated microorganism and fungi, as compare with standard drugs. The activities of these extracts are found to be quiet comparable with the standard antibiotics screened under similar conditions. So these extracts can be used as an external antiseptic in prevention and treatment of bacterial infections. The present study justified the claimed uses of leaves in the traditional system of medicine to treat various infectious disease caused by the microbes. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Further studies which aimed at the isolation and structure elucidation of antibacterial active constituents from the plant have been initiated.

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