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Comparative anti-microbial activity against leaf extract of *Ipomoea pandurata* linn and *Andrographis echioides* linn

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Abstract
To collect medicinal plants from Kolli Hills and study their Ethnobotanical view. From, the medicinal plants to determine the bacterial and fungal growth arrest assay. In this present study, the bacterial study to use Agar well diffusion method was using to detect the antimicrobial activity and fungal growth inhibition were recorded by Micro-broth dilution methods. In the study maximum inhibition was observed with methanol extracts against *Escherichia coli*. From the fungal inhibitory highest activity was recorded on *Ipomoea pandurata* Linn. On *Aspergillus Niger* when compared to *Andrographis echioides* Linn. Against *Rhizoctonia solani*. Medicinal plants have been planted long before a man can leave his feet on earth. The World health organization approximations that about 80% of the people still be subject to upon plant herbal medicines for the treatment of various infections due to easy convenience with minor side effects. The study was proved that the medicinal plants were targeting to cure microbial disease.

Keywords: bacteria, medicinal plants, kolli hills, fungi

1. Introduction
Plants valuable medicinal have been used for many human-related diseases for millennium years and in worldwide. Hence, the researchers have developed to safer medicines and bioactive compounds isolated from plant species used in herbal medicines for the invention of novel drugs [1]. Since many medicinal plant species to have pharmaceutical properties and secondary metabolites that could be used to fight for the human disease causing pathogens. Recently many bacterial and fungal strains that have developed resistance to classical antibiotics for the reasons need to address novel drugs for against pathogens and Some of the drugs from antimicrobial protein produced by animals, plants, and microorganisms [2, 3]. The Antibacterial active compounds not only microorganisms recent result from the fluids of animals, including pancreatic juice, saliva, tears, and respiratory fluid. The antibacterial activities of gastrointestinal SCFs against pathogens in the food, and related to animals destined for human consumption [4,5]. The plant therapeutic and phytopharmacognacy activity had used as old as human civilization Local people have been used medicinal herbs long back ago throughout the world to treat a variety of complaints these have exhibited clear phytomedicinal activities. Modern era new drugs from natural source such as lower and higher plant derived drugs are play a vital role against dangerous diseases including cancer, diabetes, malaria, Cardiovascular diseases and human neural diseases [6].
The medicinal plant has been producing bioactive secondary metabolites, phenolic compound, some essential oils which have potentially established known insecticidal and antimicrobial compound from the source and used for pharmaceuticals and natural therapies usage [7]. Plants have proven to be a novel, bioactive compounds against bacteria, insects, fungi weather the uniqueness evolved from millions of years ago and their ethno pharmacological functional properties have been tested for a preliminary medicinal source for those days. [8, 9].
Flavonoids from phenylalanine, which shows a large spectrum of biological activities and some of the flavonoids were reported recently. The sensitivity of bacterial strains to various flavonoids, to be separated by the spectrum of sensitivity or mechanisms of their action were firm, Therefore, investigate to be a sensitivity of various bacterial strains to flavonoids and principles of mechanism(s) against antibacterial compounds [10]. The hydrophobic attraction between bacteria and host mechanism is still unclear. The humoral responses to over whel med the pathogens that naturally occur phenomenal in the marine habitat. The molecules for the non-self-recognition and cell to matrix connections, plant active molecule known of carbohydrate-binding proteins and agglutinate with cells over carbohydrates connected with the cell surface [11].
A most of the medicinal plants have been valuable compounds of natural antimicrobial compounds and this would lead to problematic bacterial infections. According to the (WHO) report, medicinal plants would have kept itself a variety of valuable drug molecules [12,13]. Most of the medicinal plants would have synthesized valuable phytochemicals which would lead to the produced most valuable drugs in the society. Medicinal plants having a different kinds of secondary metabolites like tannins, alkaloids, phenolic compounds, and flavonoids, which have been reported high antimicrobial properties found *in vitro* conditions [14].A variety of Phytotherapy literates has been describing causes of incurable disease like infectious diseases as urinary tract infections, gastrointestinal disorders, respiratory disease, and cutaneous infections remedies through medicinal plants [15]. Past few decades, commercially synthesized drug which harmful to the human due to side effect but resistant to the microorganism [16]. Aromatic medicinal plants, which would lead to

phototherapy and food preservation. Today world scenario much-needed antibiotics have involve break the bacterial toxic mechanism. Gram-positive bacteria mainly responsible for many diseases such as post infections and food poisoning [17]. Gram-negative bacteria causes urine tract infection because its present in human intestine [18]. From the medicinal plants have multiple resistances to control different kinds of bacterial infections. The antibiotic resistance drug has multifactorial involved such as the relationship of bacteria to antibiotics, the chemical nature of the antibacterial agent, host characteristics and environmental factors [19]. The many researchers have to develop novel antimicrobial drugs and novel chemotherapeutic agents, the synthetic drug cost of production too high for this overcome adverse effects compared to plant-derived drugs and used as biological control agents [20, 21]. Even though some medicinal plants have not too many applications itself, but still the many pharmacologists produced the number of new antibiotic drugs in the industry for the past few decades. Generally, bacteria are genetically transmitted resistance to drugs to the next level so that, which are used in medical therapeutic treatment. The present study to evaluate antimicrobial agents from two different plants.

2. Material and Methods

2.1. Collection of Medicinal Plants

The medicinal plants were collected from Kollli Hills and the plants were identified according to Flora of the Presidency of Madras (1915 edition) (Figure. 1).

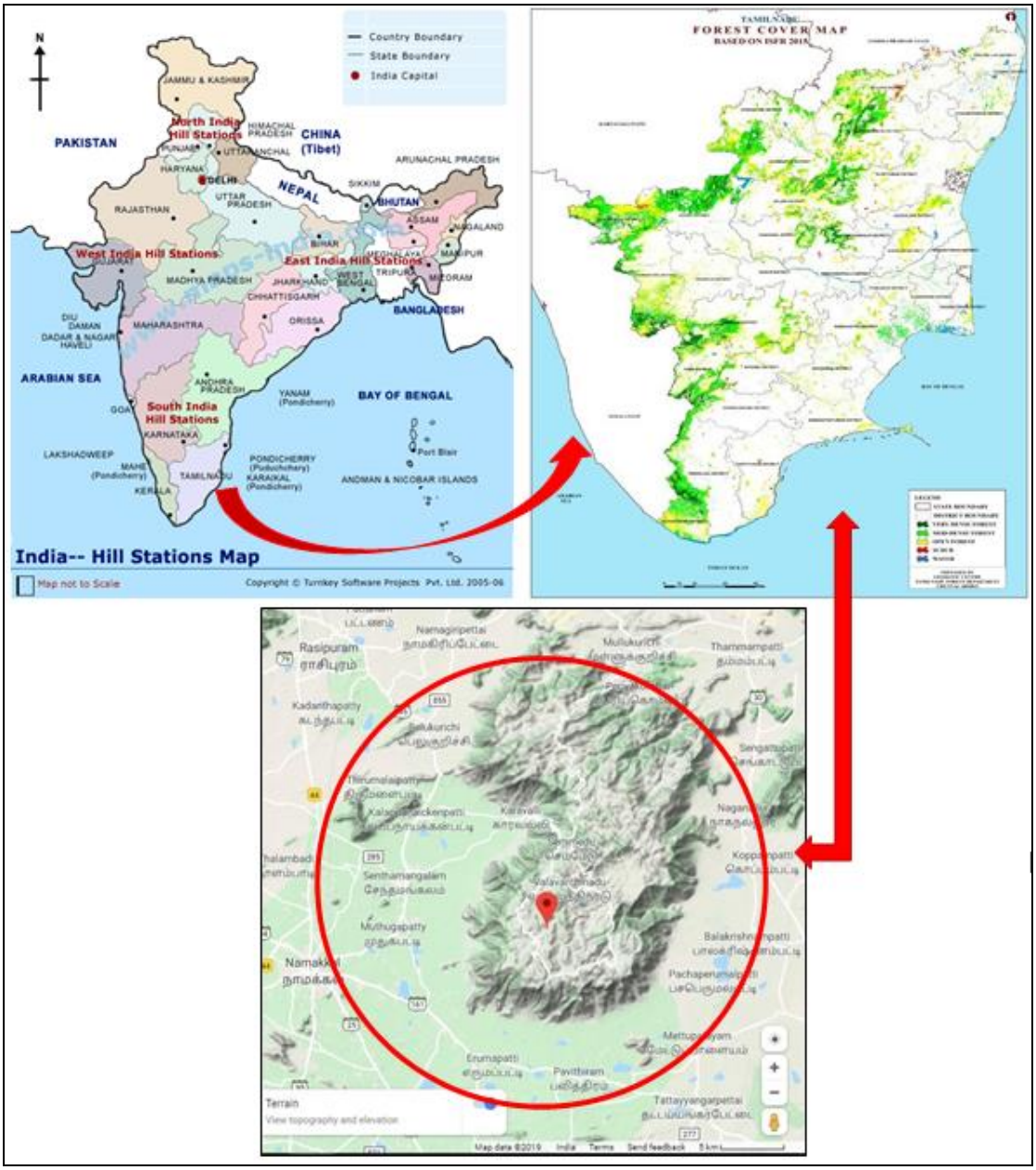

Fig 1: Study areas in Kolli hills, Eastern Ghats of Tamil Nadu, India

2.2 Preparation of Plant Extract

The collection from the source plant leaf was washed with tap water for 2-3 times and finally with distilled water, followed by methanol wash, then dried overnight at 50ºC and finally milled into a coarse powder. Soxhlet was extracted with methanol and aqueous (24 hours each) for 100gm of powdered content. All the extracts under reduced pressure are evaporated using a vacuum. All plant extracts are stored at room temperature for further use in sterile brown bottles.

2.3 Microorganisms
Human pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* are collected from the Advanced Studies Center in Botany, Madras University, Guindy Campus, Chennai.
The human pathogens such as *Aspergillus Niger* and *Rhizoctonia solani* have been obtained from the Center for Advanced Studies in Botany, Madras University, Guindy Campus, Chennai.

2.4 Culture Maintenance
The bacterial culture was Nutrient agar Media and the fungal culture was maintained by potato dextrose agar medium under aseptic conditions.

2.5 Agar Well Diffusion Method
Agar well diffusion method was followed to determine the antimicrobial activity against different plant extracts. Nutrient agar medium (NA) plates were swabbed by using a sterile cotton swab with 6 hours old mother bacterial culture of respective bacteria. The bacterial culture wells were maintained at 10mm diameter and about 2cm by using a sterile cork-borer. Methanol and water prepared a stock solution of each plant extract at a concentration of 1 mg/ml in different plant extracts. Different concentrations of solvent extracts from each plant have been added to the wells with a sterile syringe and allowed to spread at room temperature. Control experiments were served as without plant extract were set up. The plates were incubated at room temperature for 24 hours for bacterial

2.6 Calculate the Bacterial Inhibitory Ratio
The inhibition area diameter (mm) was estimated and the index of operation was determined as well. For the average values, each experiment was held at triplicates. The inhibitory activity of the bacteria was reported.

2.7 Micro-broth Dilution Antifungal Assay
Plant pathogens inhibitory behaviors by micro-broth dilution assays. To ensure sterility, all procedures were performed. In sterile 96-well microtiter plates, the broth dilution assays are performed. The broth suspension consisted of fungal spores suspended in half-power PDB, of which 90µL was applied to the wells so that each well had 2000 spores in total [22].
Each plant pathogen dissolved in 15% methanol to a concentration of 1 mg/ml. Plant extracts were diluted to a concentration of 1 mg/ml in 20 percent methanol and double dilution series were generated with half-power PDB. Antibiotic (10µL) was then applied to the suspended broth-containing wells. All wells had 100µL of the final volume. All sample plates are firmly lined with tinfoil, parafilm sealed and 48 hours incubated at 23-25°C. Micro-broth dilution antifungal assay was spectrophotometrically determined at 595 nm using a Bio-Rad microtiter plate reader.

2.8 Data Processing
To calculate the percentage of fungal growth inhibition by using the following formulae, [23].

$$\text{Growth inhibition (\%)} = 100 - \frac{100 \times (\text{A 595 of well} - \text{Average A595 of background})}{\text{Average A595 of growth wells} - \text{Average A595 of background}}$$

3. Results & Discussion
3.1. Collected Medicinal Plants
Survey of medicinal plants on rare plants were collected from Kolli hills. Based on the ethano-pharmacology effects with the help Nadukombai village local peoples the plants (Table 1) were collected. Totally, wild tuberous medicinal plants are arranged in alphabetical order with their botanical name.
Vernacular name, family, and parts used medicinal uses (Figure 2 -3).

Table 1: Medicinal plants of Kolli hills from Nadukombai village

S No.	Name of the Species	Family	Name	Parts used	Medicinal use
1	Cassia tora	Leguminosae	Sickle Senna	Root	laryngitis, rheumatism, diseases of the spleen, ring worm, scabies, etc.,
2	*Ipomoea pandurata*	Convolvulaceae	Wild potato vine	Root	Asthma, beginning stages of tuberculosis; "blood purifier"; powdered plant used in tea for headaches, indigestion.
3	*Eclipta alba*	Asteraceae	False daisy	Whole plant	Bitter, acrid, thermo genic, alterative, anti- inflammatory, anthelmintic. Anodyne, vulnerary, ophthalmic, digestive, carminative, haematinic, diuretic, aphrodisiac, trichogenous, debostruant, depurative and febrifuge.
4	*Leucas aspera*	Lamiaceae	Thumbai	Leaves, Root	Treatment of respiratory tract disorders, edema, gastrointestinal disorders, pain, and etc.,
5	*Andrographis echioides*	Acanthaceae	False Water willow	Whole plant	To cure various diseases like goiter, liver diseases, fertility problems, bacterial, malarial and fungal disorders and decrease the falling and graying of hair
6	*Pavonia zeylanica*	Plumbaginaceae	Ceylon Swamp Mallow	Leaves, Root	Skin disorders and intestinal worms

Fig 2: A. *Cassia tora* Linn. (Family: Leguminosae); B. *Ipomoea pandurata* (L.) G. Mey (Family: Convolvulaceae); C. *Eclipta alba* Linn. (Family: Asteraceae).

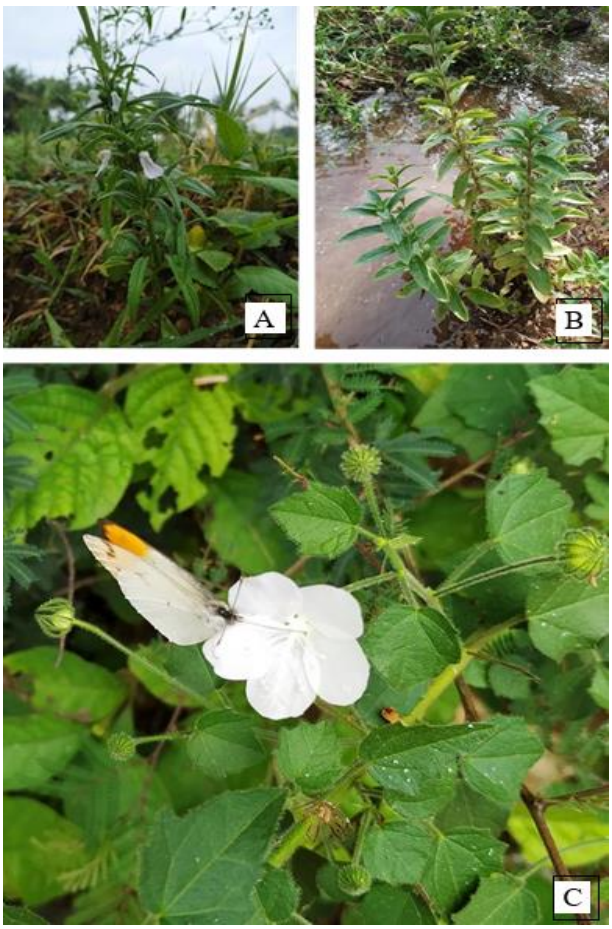

Fig 3: A. *Leucas aspera* Linn. (Family: Lamiaceae); B. *Andrographis echioides* Linn. (Family: Acanthaceae); C. *Pavonia zeylanica* Linn. (Family: Plumbaginaceae)

3.2. Bacterial Inhibition Activity

Table 2 shows that the bacterial inhibition activity. The highest inhibition of methanol was extracted from *I. pandurata* and *A. echioides.* The two bacteria were used for determining the growth inhibition assay. The bacterial growth arrest were starting from 25mg/ml on *E. coli* meantime, *S. aureus* bacterial growth was arrest at 75mg/ml against *I. pandurata* (Figure 4). The reduction of bacterial growth was confirmed by the antimicrobial activity of plant extracts. The plant extracts containing, secondary metabolites and its release from the films killed the bacteria by either breaking the cell walls using electrostatic attraction between the positively charged and negatively charged bacterial cells. In the molecular pattern, destroying the DNA as well as proteins metabolism of the bacterial cells [24]. The mechanism of bacterial cell wall was damage through biological substance [25]. The results of the bacterial nucleic acid expression were switched off and it's going down the regulation process. Compared to the water as a control, the bacterial cell morphology (control) and the treated bacterial cells morphology showed shrunken internal content and distorted cell walls, and established of the cell leakage [26]**.**

Table 2: Bacterial growth inhibition on *Escherichia coli* and *Staphylococcus aureus* against *Ipomoea pandurata* and *Andrographis echioides*

Concentration (mg / ml)	Bacterial inhibition against *I. pandurata* (mm)		Bacterial inhibition against *A. echioides* (mm)	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*
25	8	-	2	-
50	14	-	4	-
75	21	3	5	-
100	29	5	7	2
Control (H_2O)	-	-	-	-

-No activity

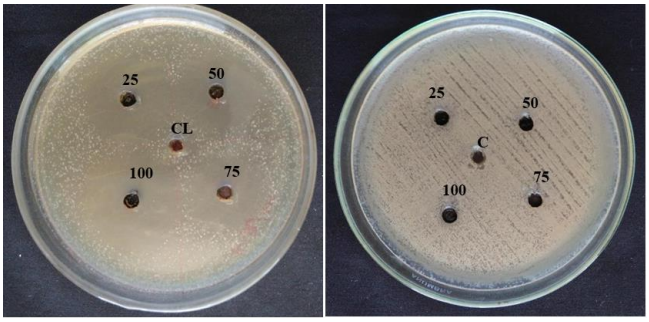

Fig 4: Bacterial growth inhibition on *Escherichia coli* and *Staphylococcus aureus* against *Ipomoea pandurata Linn.*

On control was no growth inhibition against tested bacteria. From *A. echioides* extract on *E. coli* was started from 25mg/ml, however *E. coli* and *S. aureus* growth was arrest on 100mg/ml at low level/no activity (Figure 5). The morphology of the disc diffusion test shown in and around leaking cells is plant extracts that damaged the cell walls, entered the cells and caused cell leakage. The gram-positive (*E. coli*) and gram-negative (*S. aureus*) culture against plant extract their inside of the cytoplasm may destroy the DNA and protein. Medicinal plants proved that cure various human disorders in the form of partially purified secondary metabolites [27]. The results of two plant extract, revealed that the maximum inhibition on gram-positive and followed

by gram-negative bacterial strains, which were sensitive growth arrest as previous report at 25-500 µL/mL [28].

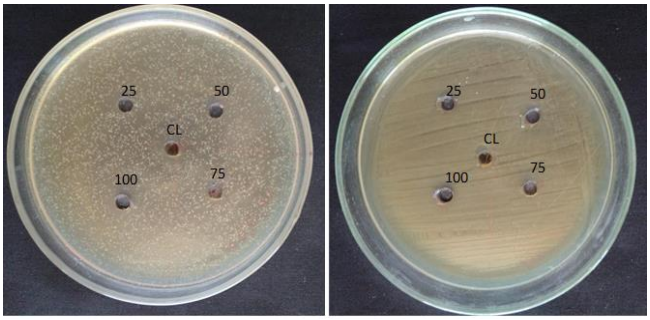

Fig 5: Bacterial growth inhibition on *Escherichia coli* and *Staphylococcus aureus* against *Andrographis echioides Linn.*

3.3 Fungal Inhibitory Activity

Figure 6 indicates that, *I. pandurata* extracts had a total average maximum inhibitory concentration range at 80mg/ml which is considered high active, fungal growth inhibition on *A. Niger*. *A. echioides* extracts had highest activity against on *A. niger* and *R. solani*. Antifungal activity of *I. pandurata* against *R. solani* was measured in different concentrations (10 – 100 mg/ml) of plant extracts. Table 3 showed that, the percentage of Inhibition (%), at 10 mg/ml of *I. pandurata* extract in range is 0.61%. The concentration was increased the fungal activity range was increased.

Table 3: Antifungal activity of *Ipomoea pandurata* and *Andrographis echioides* against *Aspergillus Niger* and *Rhizoctonia solani*. The effects of different concentrations (10 – 100 mg/ml) of plant extracts dissolved in DMSO are shown.

Concentration (mg/ml)	***I. pandurata* (%)**		***A. echioides* (%)**	
	A. niger	***R. solani***	***A. niger***	***R. solani***
10	0.28	0.61	0.29	01.21
20	04.06	03.70	04.19	05.48
30	16.23	11.11	14.37	11.89
40	31.01	24.07	32.63	25
50	35.65	30.55	38.62	31.70
60	41.44	36.41	44.31	36.89
70	48.40	42.28	51.79	43.29
80	55.94	49.07	60.47	48.17
90	61.44	56.17	63.47	57.31
100	66.37	61.11	69.46	64.63

The fungal growth was not detect/no inhibition in blank. Serial concentrations of methanol extracts were maintained against the fungal culture, the concentrations at 10-100mg/ml in the different well was served. *A. echioides* extract on *A. Niger* was highest activity when compared to the other plant extracts. This confirms the low toxicity of acetone as a solvent for antifungal bioassays [29]. The tetracycline was used as positive control and the minimum inhibitory concentration value of 17.8µg/ml against plant extracts. From this previous report to support our present studies fungi against the plant extracts with minimum inhibitory concentration values of 0.46 mg/ml against *R. solani* and *P. ultimum.*

According to Filimonov et al, [30]. HOIL produces fungal endophytes belonging to the *Aspergillus* spp. Endophytic fungi metabolite such as astellatol was isolated from *Aspergillus variecolor*. The astellatol compound is rare 5 position carbon framework sesterterpenoid. The bioactive molecules more active against different gram-positive and gram-negative bacteria.

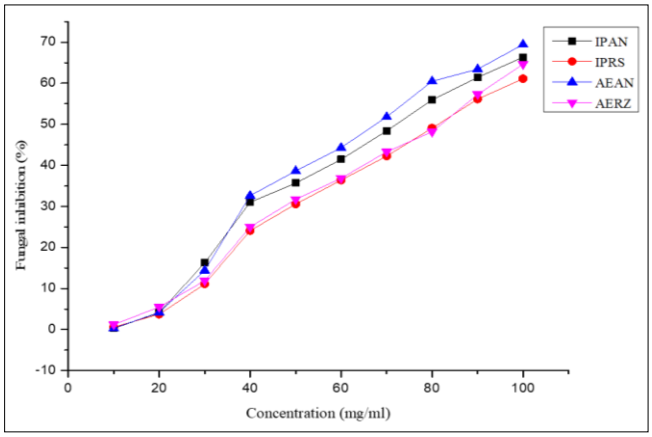

Fig 6: Fungal growth inhibition against crude extract of *Ipomoea pandurata* and *Andrographis echioides.* Here, IPAN - *Ipomoea pandurata* with *Aspergillus Niger*; *IPRZ - Ipomoea pandurata* with *Rhizoctonia solani;* AEAN - *Andrographis echioides* with *Aspergillus Niger*; AERZ - *Andrographis echioides* with *Rhizoctonia solani.* The effects of different concentrations (10-100 mg/ml) of plant extracts dissolved in DMSO are shown.

4. Conclusion

Plant leaf extracts are a potentially useful source of antimicrobial compounds. In the study, *E. coli* is high ranges were inhibited plant leaf extracts. From the *A. Niger* fungal inhibitory highest activity was recorded on *I. pandurata* when compared to *A. echioides* against *R. solani.* The current scenario we faced natural antibacterial drugs has been increasing resistance of bacterial strains to a variety of different agents.

However, explaining mechanisms of action and resistance of bacteria to these agents may provide basic evidence for microbiological research; this would be useful for understanding the fundamental cellular processes.

Conflicts of interest

None.

Source of funding

None

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