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ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF *TRICHOSANTHES CUCUMERINA* L AN ENDANGERED ETHNOMEDICINAL HERB

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Key words:

Antibacterial activity, *Trichosanthes cucumerina*, different extracts, Zone of inhibition.

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ABSTRACT: The present work has been under taken to study the antibacterial activity of stem, leaf, flower and seed extracts of *Trichosanthes cucumerina* L against disease causing bacteria. Antibacterial activity of different solvent extracts (Aqueous, Methanol, Chloroform, Petroleum ether, Acetone) of stem, leaf, flower and seed of *T. cucumerina* has been studied to find out their activity against nine pathogenic bacteria viz., *Bacillus cereus, Micrococcus luteus, Escherichia coli, Proteus vulgaris, Klebsiella pneumonia, Bacillus sphericus, Salmonella typhimurium, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The antibacterial activity of the extracts of different plant parts was done through well diffusion method and by measuring the inhibition zone around the disc. According to our observations, the seed extracts of *T. cucumerina* exhibited antibacterial activity against all the bacteria under study. The results provided evidence that the species *T. cucumerina* can be used as a potential source of antibacterial agent.

INTRODUCTION: Medicinal plants are of great importance to the mankind and their value lies in chemical substances that are present in them. Most of them are physiologically active substances with beneficial activities when utilized in adequate quantities. In recent years, there has been growing concern in the usage of secondary metabolities or phytochemicals with pharmacological activities for the benefit of health and well being of human as well as animals. Thus it is interesting to know that plant bioactive substances are sequence of medicinal agents and their antibacterial efficacy can also be used in the treatment of bacterial infections.

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Considering the rich diversity of Indian medicinal plants it is expected that, the screening of plant extracts for antibacterial activity may be beneficial for human and plant diseases.

The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents. Infectious diseases account for high proportion of health problems in the developing countries [1]. Resistance to antibiotics emerges in bacteria due to genetic mutations and consecutive selection of resistant mutants through selective pressure of antibiotics present in large amount in soil, plants, animals and humans [2]. Problems with drug resistant micro organisms and side effects of modern drugs and emerging diseases where no medicines are available have stimulated renewed interest in plants as significant source of new medicines [3]. Traditionally used medicinal plants produce a variety of compounds of known

therapeutic properties. These substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs [4].

The Cucurbitaceae plants especially seeds show the antibacterial properties [5]. Hugo *et al*., [6] investigated the antibacterial activity of methanol extract and ethyl acetate mixture of stem and leaf of *Coccinia indica* against *Pseudomonas syringae* and *Staphylococcus aureus*. Investigations were carried out on methanol extract of *Coccinia* leaves by Deewanjee *et al.,* [7] against selective gram positive and gram negative bacterial strains.

In vitro antibacterial activity of leaves and stem extracts of *Coccinia grandis*, was investigated against four gram positive and six gram negative bacteria using water, hexane and ethyl acetate extracts by Umbreen *et al.,* [8].

Antimicrobial activities of *Momordica charantia, Mentha piperata* and *Pisum sativum* were reported by Subhan and Tariq [9]. Ethanol extracts from leaves of *Cayaponia podantha* (Cucurbitaceae) and four other Brazilian plants were screened for antimicrobial activity by Maria *et al.,* [10]. Alessandra *et al.,* [11] studied the chemical composition and antimicrobial activity of *Momordica charantia* seed essential oil. *In vitro* antimicrobial activity of fruit and leaf extracts of *Momordica charantia* was investigated by Mwambete [12]. Similar work was done by Belsem *et al.,*[13] on Tunisian, *Citrulllus colocynthis*.

In recent times, the rapid development of multi drug resistant bacterial and fungal strains of clinically important pathogens fetches the interest of scientists to develop newer broad spectrum antimicrobial agents. The less availability and high cost of new generation antibiotics necessitates looking for the substances from alternative medicines with claimed antimicrobial activity. These developments and associated increase in microbial infections intensified the search for new, safe and more efficacious agents to combat serious microbial infections.

There appears to be no evidence in the literature of the antibacterial activity of organic extracts (flower and seed) of *T. cucumerina* against gram-positive and gram negative bacteria though there are reports on leaf extracts. According to our literature survey no such investigations reported on the petroleum ether, chloroform and acetone extracts of leaf, stem, flower and seed of *T. cucumerina* against gram positive and gram negative bacteria. Therefore, the findings here are the first report of antibacterial activity of these extracts.

The present investigation has been attempted to evaluate antibacterial activity of different solvent extracts of various parts of *T. cucumerina* using some human pathogenic bacteria.

MATERIAL AND METHODS:

Microorganisms used:

The following gram positive and gram negative pathogenic cultures of human and plants were used for testing the antibacterial activity of plant extracts. These cultures were obtained from different sources listed below and maintained on their respective media.

Bacterial cultures:

The following cultures were obtained from Institute of Microbial Technology (IMTECH), Chandigarh and Department of Microbiology, Kakatiya University, Warangal (TS).

Salmonella typhimurium	KUCC 14
Micrococus luteus	KUCC 09
Bacillus cereus	KUCC 23
Escherichia coli	KUCC 03
Klebsiella pneumonia	KUCC 11
Proteus vulgaris	KUCC 21
Staphylococcus aureus	MTCC 96

Media used for the assay:

The following growth medium is used to culture the microorganisms.

Nutrient agar medium (for 1L):

Peptone	5.00g
Beef extract	3.00g
Sodium chloride	5.00g

Agar	20.00g
Distilled water	1000 ml

Preparation of sample/test solution for antibacterial activity:
A concentration of 250 mg/ml of each solvent extract of different plant parts was prepared in DMSO (which did not influence the microbial growth).

Plant material:
The plant material (stem, leaves, flowers and seeds) of *T. cucumerina* was collected from the research field, Department of Biotechnology, Kakatiya University, Warangal, Telangana State, India. The collected plant material was washed thoroughly with distilled water to remove the surface contaminants and then dried under room temperature for 30-45 days. Later finely powdered using an electric blender and stored in air tight containers for future use.

Preparation of the extracts:
The shade dried powder (25gm) was used for the extraction with 150 ml 80% methanol for 24 hours by Soxhlet equipment and filtered through 0.45 µm membrane filter. This filtrate was evaporated under reduced pressure and dried in a rotator evaporator at 55°C. Dried extracts were stored in screw cap bottles at -20°C and used as stock. Further, the same was diluted by using distilled water to arrive at different concentrations (1:1, 1:2 and 1:3). Fresh extracts were prepared for every third day.

Cultivation techniques:

a) Plant preparation: Agar slants were prepared by dispensing 10 ml of aliquots of molten medium into 30 ml culture tubes and sterilized. The culture tubes were then laid on a 30 angle and allowed to set.

b) Plate preparation: Using sterile technique, 20ml aliquots of sterile molten medium were transferred to sterilized petridishes. After solidifying, the plates were used for the assay.

c) Sub-culturing: Subcultures were prepared by transferring loopful of inoculum from culture slants to freshly prepared agar slants. These were incubated in the desired conditions.

Preparation of inoculums:
By the standard method of inoculation [14] an inoculating loop was touched each of four or five well isolated colonies of the same morphological type and inoculum was inoculated into 5ml of nutrient broth. The broth cultures were allowed to incubate at 37^{o}C for 24 hrs until a slight visible turbidity appeared. The turbidity of actively growing broth cultures was then adjusted with broth to obtain a half of MC Farland standard (1×10^{8} to 5×10^{8} cfu/ml). This was used as a starting inoculum for the assay.

Antimicrobial assay by agar well diffusion method:
The antimicrobial assay was carried out by agar well diffusion or agar cup plate method [15].

Agar cup plate method:
A standardized 1 to 2×10^{4} cfu 0.5ml MC Farland standards was introduced onto the surface of sterile agar plate and evenly distributed the inoculum by using a sterile glass spreader. Simultaneously 8 mm wells were cut from the plate using a sterile cork borer.

50µl of extract at a concentration of 200 mg/ml was introduced into each well. The agar plates were incubated aerobically at 37^{o}C. After 24hrs, the inhibition zones were measured with a ruler and compared with the control well containing only DMSO and 10mg/ml of Streptomycin as standard.

Data analysis:
All the tests were conducted in triplicates. The data of all the parameters were analyzed.

RESULTS:
The results obtained from the investigations showed that almost all the extracts exhibited considerable antibacterial activity against gram positive and negative bacteria. The obtained data on stem, leaf, flower and seed are presented in **Table 1** and in **Fig. 1-4.**

Antibacterial activity of stem extracts:
The petroleum ether extracts was most effective and showing maximum inhibition zone of 25mm against *Bacillus sphericus* **(Table 1; Fig.1).** Acetone extracts were also shown maximum effect

(inhibition zone-23mm) on *Pseudomonos aeruginosa* followed by *Staphylococcus aureus, Proteus vulgaris* and *E.coli* (**Plate 1**). Whereas less effect was found with aqueous extracts against *B. cereus* and *E. coli* and the same extract didn't show effect on *Micrococcus luteus.*

TABLE 1: ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF *T. CUCUMERINA* AND STANDARD DRUG BY AGAR WELL DIFFUSION METHOD

	Plant extracts																							
	Stem						Leaf						Flower						Seed					
Test organism	**W**	**M**	**C**	**PE**	**A**	**S**	**W**	**M**	**C**	**PE**	**A**	**S**	**W**	**M**	**C**	**PE**	**A**	**S**	**W**	**M**	**C**	**PE**	**A**	**S**
Bacillus cereus	10	18	11	15	11	31	21	13	22	12	23	33	10	18	11	15	11	31	19	19	14	22	11	31
Micrococcus luteus	-	12	11	12	11	32	22	15	17	15	22	32	19	19	14	22	11	35	13	15	11	16	31	33
Escherichia coli	10	22	18	22	21	33	24	21	21	19	24	36	14	21	22	25	11	33	15	15	11	15	32	33
Proteus vulgaris	15	12	22	21	22	32	20	25	21	21	16	36	14	15	21	22	16	36	21	22	18	21	33	35
Klebsiella pneumoniae	19	19	14	22	11	35	21	25	23	24	21	33	21	13	22	12	23	33	25	25	22	22	32	35
Bacilllus sphericus	14	21	22	25	11	33	21	25	24	26	21	35	21	13	22	12	23	33	25	25	14	15	35	33
Salmonella typhimurium	14	15	21	22	16	36	25	21	25	21	24	35	22	15	17	15	22	32	25	25	22	11	33	30
Pseudomonas aeruginosa	21	13	22	12	23	33	25	25	24	21	21	35	24	21	21	19	24	36	21	21	21	16	36	33
Staphylococcus aureus	15	23	22	21	22	38	22	21	21	23	21	38	22	24	25	22	12	33	25	25	22	23	33	35

Showing the zone of inhibition (in mm)**, W**-Water, **M**-Methanol, **C**-Chloroform, **PE**-Petroleum.ether, **A**-Acetone, **S**-standard (Streptomycin)

PLATE 1:

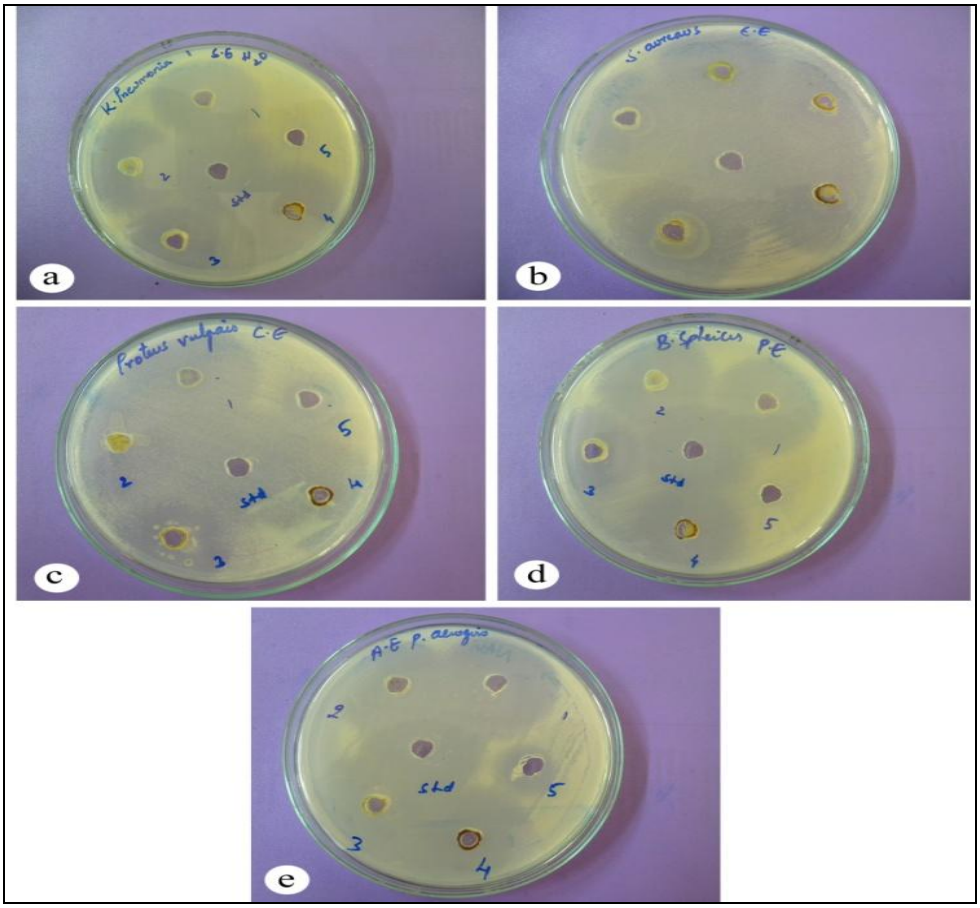

FIG. a-e: SHOWING THE ZONE OF INHIBITION OF: **a)*Pseudomonas aeruginosa b) Staphylococcus aruerus c) Proteus vulgaris d) Bacillus sphericus e) Pseudomonas aeruginosa* against various solvent extracts of stem of *T. cucumerina*(Std=Standard; 1=Water; 2=Methanol; 3=Chloroform; 4=Petorleum ether; 5= Acteone).

Antibacterial activity of leaf extracts:

The petrolelum ether (PE) leaf extracts of *T. cucumerina* was found to be the most effective with maximum inhibition zone of 26mm against *B. Sphericus* **(Table 1**), followed by methnaolic extract against *Klebsiella pneumonia, Proteus valugaris, B. sphericus and Pseudomonas aeruginosa* (**Plate 2**). Acetone extract had also shown effective on *E. coli* and *S. typhimurium* (**Fig. 2**). Aqueous leaf extract had also shown effective against *S. typhimurium* and *P. aeruginosa*.

PLATE 2

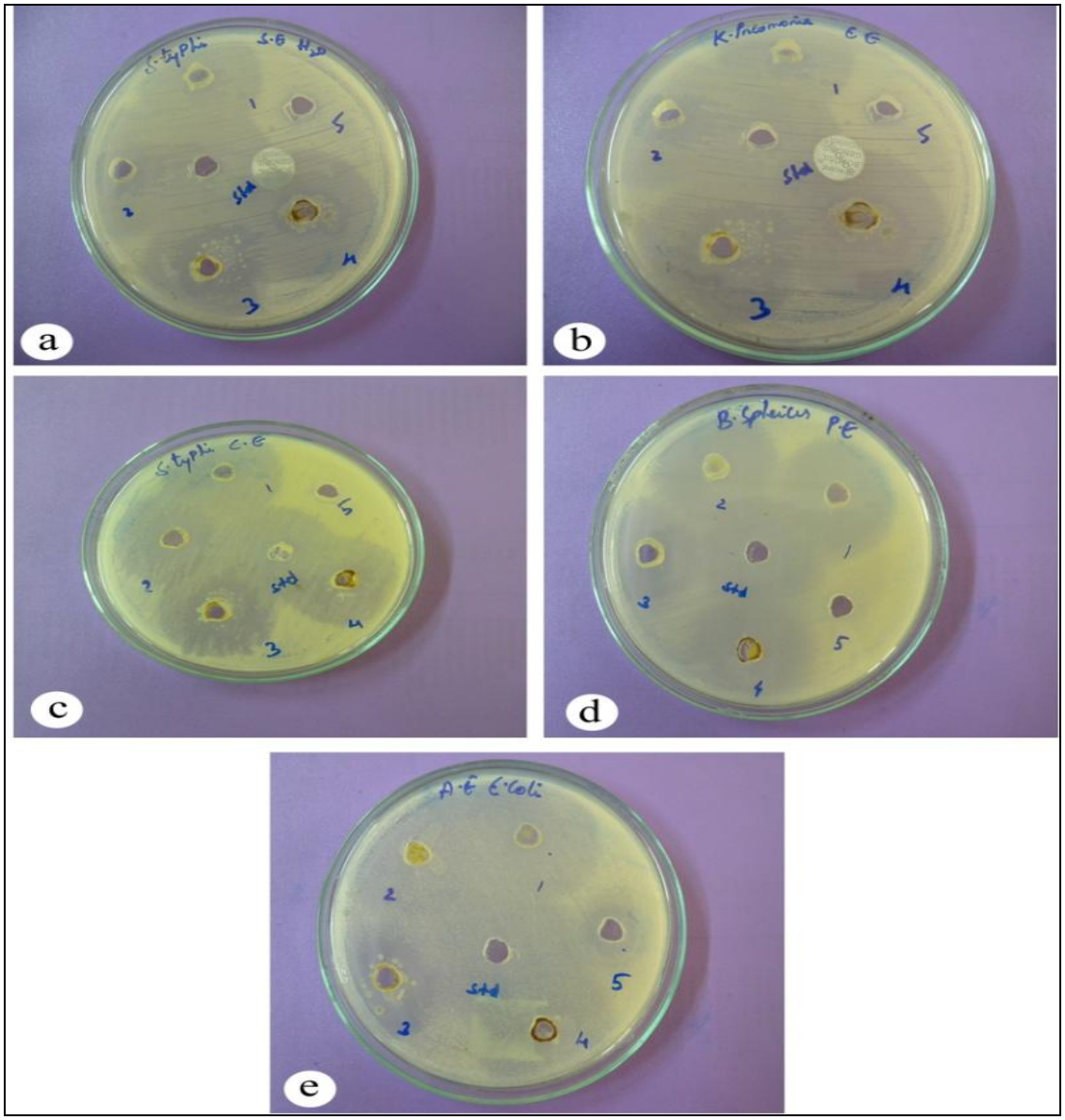

FIG. a-e: **SHOWING THE ZONE OF INHIBITION OF: a)*Salmonella typhimurium* b) *Klebsiella pneumoniae c) Salmonella typhimurium* d) *Bacillus sphericus e) Escherichia coli* against various solvent extracts of leaf of *T. cucumerina* (Std=Standard; 1=Water; 2=Methanol; 3=Chloroform; 4=Petorleum ether; 5=Acteone).**

Antibacterial activity of flower extracts:

The results on antimicrobial activity of floral extracts of *T. cucumerina* are presented in **Table-1** and shown in **Fig. 3**. The petroleum ether and chloroform extracts had shown maximum zone of inhibition (25mm) against *E. coli* and *S. aureus* respectively (**Plate 3**). The extracts with aqueous, methanol and acetone were found to be effective on *P. aeruginosa*, *S. aruerus* and *P. aeruginosa* respectively. Least zone of inhibition was observed with aqueous extracts on *B. cereus* (**Fig. 3**).

PLATE 3

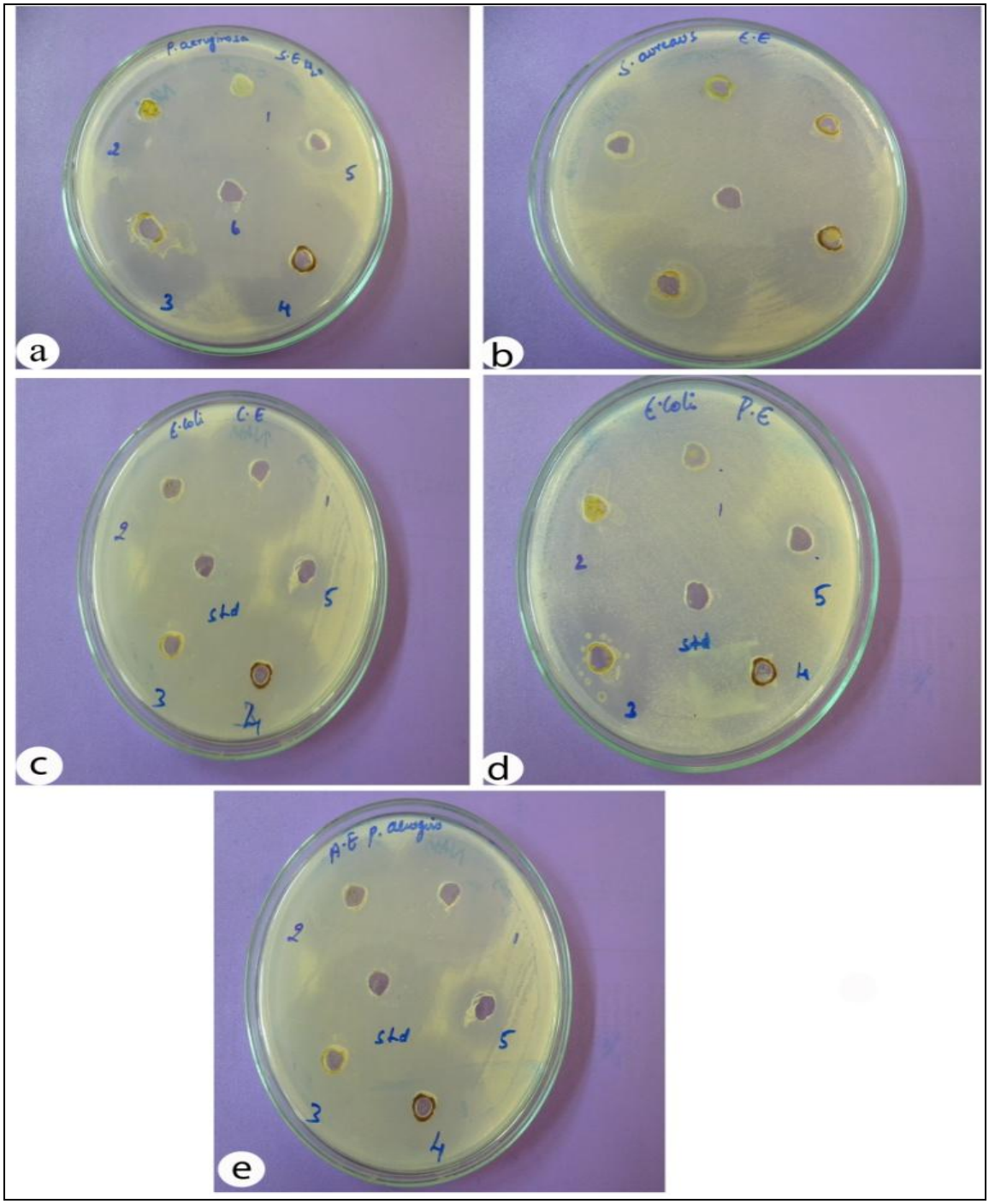

FIG. a-e: SHOWING THE ZONE OF INHIBITION OF: a)*Pseudomonas aeruginosa b) Staphylococcus aruerusc) Staphylococcus aruerus d) Escherichia coli e) Pseudomonas aeruginosa* against various solvent extracts of flower of *T. cucumerina* (Std=Standard; 1=Water; 2=Methanol; 3=Chloroform; 4=Petorleum ether; 5= Acteone).

Antibacterial activity of seed extracts

The results on various solvent extracts of seed of *T. cucumerina* on different pathogenic bacteria are presented in **Table 1** and shown in **Fig. 4**. The seed extracts of aqueous, methanolic and acetone had shown promising effect against *M. luteus, E.coli, K. pneumonia, B. sphericus, S. typhimurium, P. aeruginosa* and *S. aureus* (**Plate-4**). Whereas petroleum ether and chloroform extracts of seed showed moderate effect on micro-organisms used in the present investigations.

PLATE 4

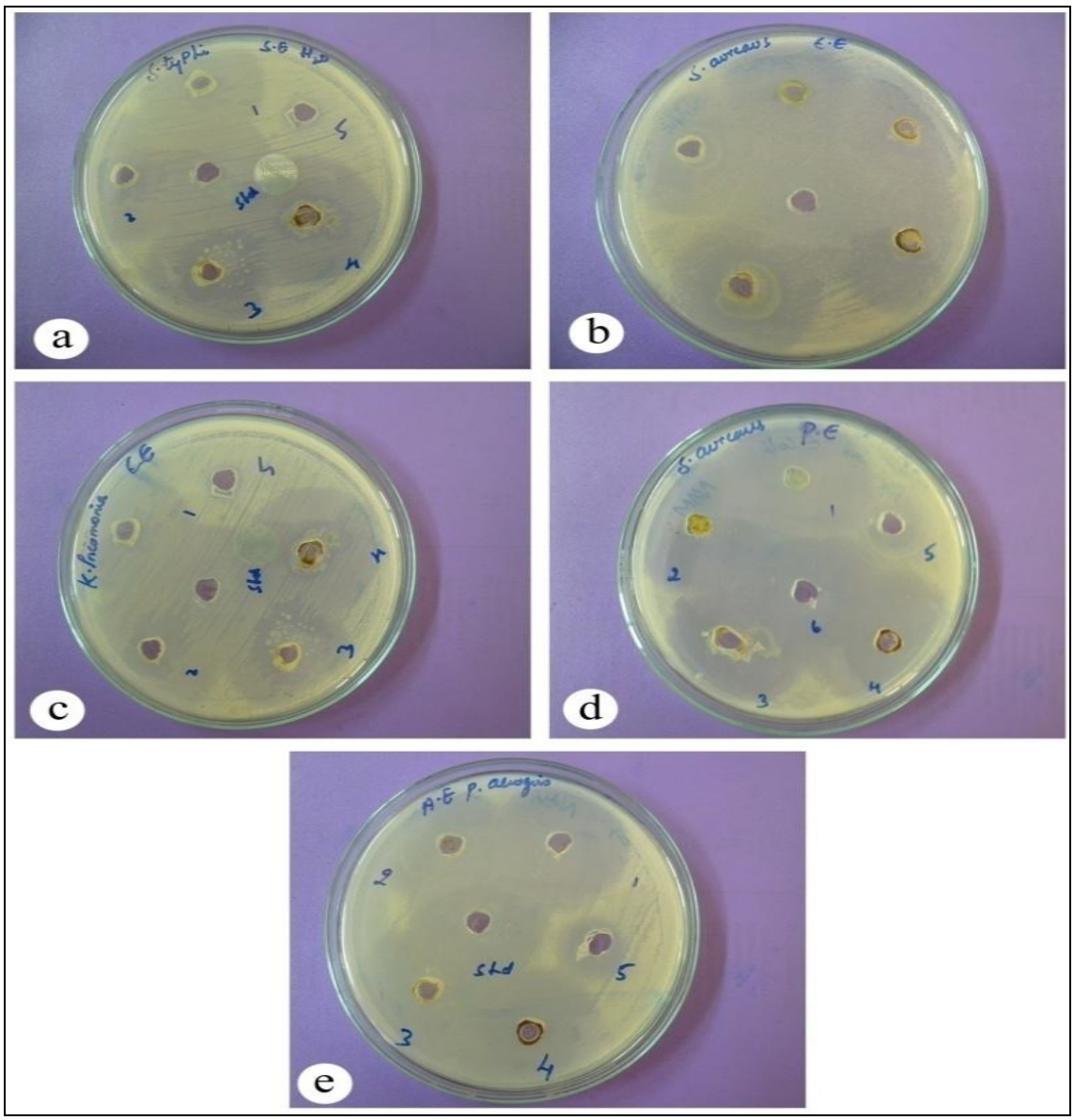

FIG. a-e: SHOWING THE ZONE OF INHIBITION OF: a) *Salmonella typhimuriumb) Staphylococcus aruerusc) Klebsiellapneumoniae d) Staphylococcus aruerus e) Pseudomonas aeruginosa* against various solvent extracts of seed of *T. cucumerina*(Std=Standard; 1=Water; 2=Methanol; 3=Chloroform; 4=Petorleum ether; 5=Acteone).

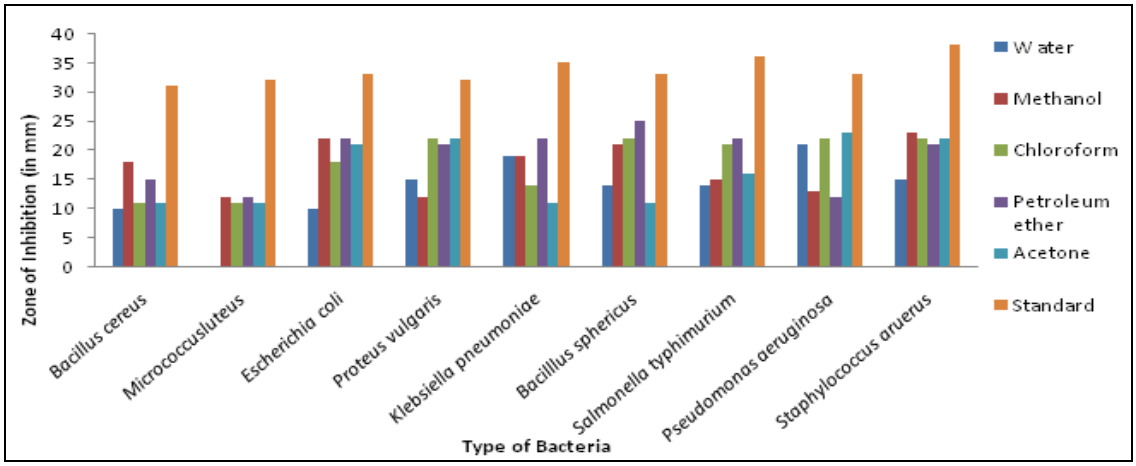

FIG. 1: ANTIBACTERIAL ACTIVITY OF STEM EXTRACTS OF *T. CUCUMERINA* AND STANDARD DRUG BY AGAR WELL DIFFUSION METHOD

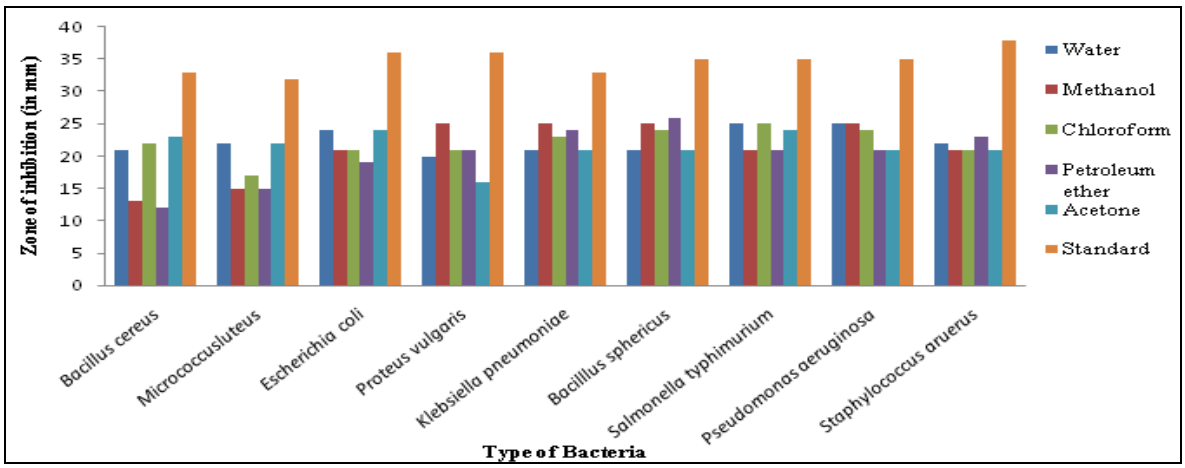

FIG. 2: ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS OF *T. CUCUMERINA* AND STANDARD DRUG BY AGAR WELL DIFFUSION METHOD

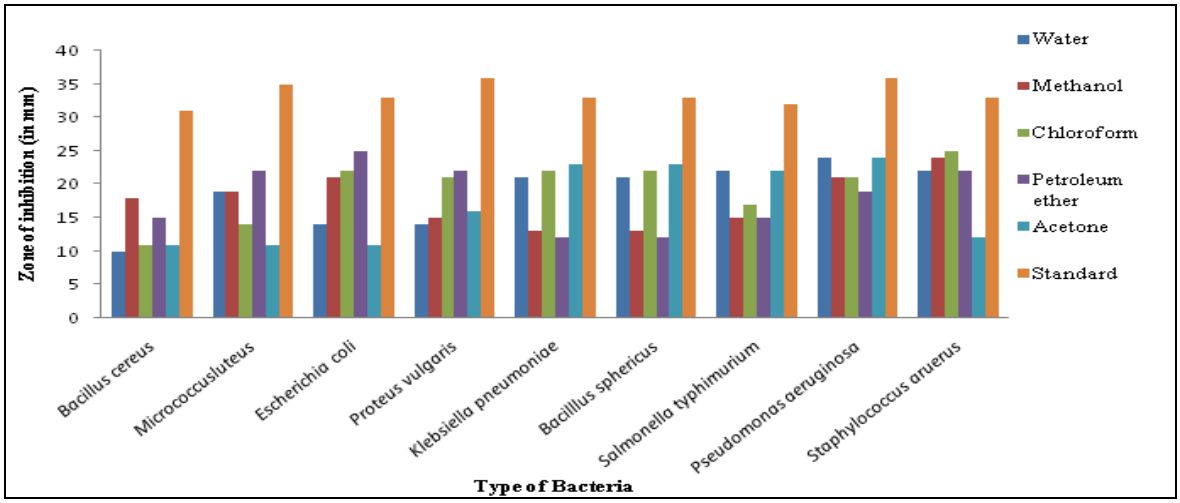

FIG. 3: ANTIBACTERIAL ACTIVITY OF FLOWER EXTRACTS OF *T. CUCUMERINA* AND STANDARD DRUG BY AGAR WELL DIFFUSION METHOD

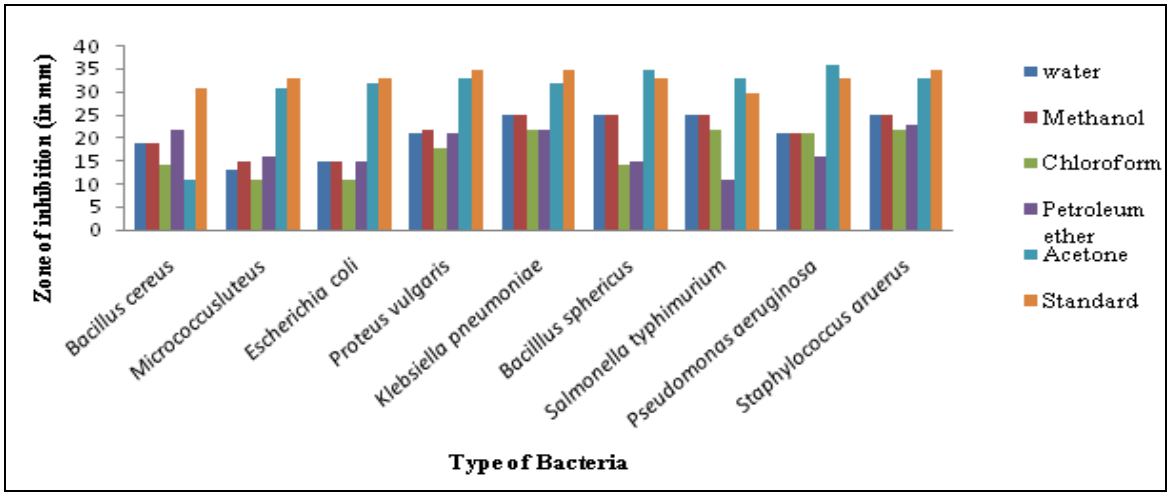

FIG. 4: ANTIBACTERIAL ACTIVITY OF SEED EXTRACTS OF *T. CUCUMERINA* AND STANDARD DRUG BY AGAR WELL DIFFUSION METHOD

DISCUSSION: In the present investigation all the parts of *T. cucumerina* plant extracts were found effective on various microorganisms but it was dependent upon the plant part and as well as the solvent used (**Table 1**). The extracts of seed with water, methanol and acetone had shown maximum effect on almost all the microorganisms tested. The seed extracts with the acetone as a solvent were

found to the best against all pathogenic bacteria tested followed by methanolic extract of leaf.

Antibacterial activity of leaves and stem of *Coccinia indica* has been detected the significant activity in methanol and ethyl acetate extracts against different bacteria and providing support to the fact that methanol is a better solvent for extraction and isolation of phytochemicals having antimicrobial activity [7, 8]. According to our observations acetone extracts of seed showed more in *T. cucumerina*.

Sumathi and Pushpa [16] and Umadevi *et al* [17] reported the inefficiency of aqueous extracts towards the tested organisms. Whereas in the present investigations, aqueous extracts of different parts of *T. cuccumerina* were found effective against pathogenic bacteria used (**Table-1**). The basis of varying degree of sensitivity of test organisms may be due to the intrinsic tolerance and the nature and combinations of phytocompounds present in the extracts[4]. Hexane, dichloromethane, ethyl acetate and ethanol extracts of leaf of *Luffa operculata* were investigated by Jagessar *et al.,* [18] against *S.aureus* and *E.coli* which proved the ethanol and ethyl acetate extracts to be very effective. Tomori *et al.,*[19] reported that *Lagenaria breviflora* ethanol extract of fruit inhibited both gram positive (*B. subtilis* and *S. aureus*) and gram-negative bacteria (*S. gallinarium, P. aeruginosa, K. pneumonia* and *E.coli*).

The prominent antimicrobial activity of ethanol extract of *Miomordica charantia* against *S. aureus* and *E.coli* was reported by Jagessar *et al* [20]. Bonyadi *et al.,* [21] reported that among leaf, stem, seed and fruit ethanolic extracts of *Bryonopsis laciniosa* tested, leaf and stem extracts exhibited antimicrobial activity against gram-positive and gram-negative bacteria and significant inhibition zone was seen against *S. aureus, Micrococcus luteus* and *B. cereus.* Whereas in the present investigations, leaf and seed extracts of *T. cucumerina* exhibited maximum antimicrobial activity.

Recently we have also reported on antifungal activity (chary *et al.,* 2015). Among them, plant extracts of different parts of *T. cucumerina.* The seed extracts were most significant with larger zones of inhibition and inhibiting a broad spectrum of fungi (*Penicillum spp, Fusarium chlmydosporum A. flavus, A. terreus, A. fumigatus* and *Curvularia lunata*) followed by remaining extracts.

The present investigations have revealed the presence of antimicrobial activity of different solvent extracts of stem, leaf, flower and seed of wild endangered species *T. cucumerina*. The species *T. cucumerina* extracts exhibited antibacterial ctivity against both gram (+) ve bacterial strains such as *B. cereus, B. sphericus, M. luteus*, and gram(-) ve bacterial strains such as *E. coli, P. vulgaris, K. pneumonia, S. typhimurium* and *P. eruginosa,* indicating the presence of a broad spectrum of antibacterial compounds present in the plant.

CONCLUSION: Thus, it is evident from our findings that the phytochemicals / bioactive compounds present in the stem, leaf, flower and seeds of *T. cucumerina* can be used not only as antifungal but also as antibacterial and also therapeutic agents to cure many diseases. Before drawing any concentration further work can be carried out in *T. cucumerina* on these lines.

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