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ANTIBACTERIAL POTENTIAL, PHYTOCHEMICAL INVESTIGATION AND CHARACTERIZATION OF ANTIBACTERIAL PROTEIN OF DIFFERENT FRACTIONS OF *ACALYPHA INDICA*

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ABSTRACT

Medicinal plants are extensively used to cure various infectious diseases in human. Our present study was undertaken to investigate antibacterial activity in leaf, stem and root extracts of *Acalypha indica* against the human pathogens such as *Staphylococcus sp, E. coli, Salmonella sp, Klebsiella pneumonia and Pseudomonas*. The shade dried leaves, stem and root powder was used to prepare extracts by using and acetone, methanol and ethanol by soxhlet method. The antibacterial activity was studied by using agar well diffusion method with different concentrations of extracts (25, 50, 75 & 100µl). The result showed that ethanol leaf extract is effective against tested pathogen. Highest inhibition of zone was recorded in *E. coli* (22 cm). Phytochemical analysis of *Acalypha indica* was done. In the present study, the antimicrobial factors of *A. indica* were found to have Flavonoids, Triterpenoids, Phenolic compounds and proteins in leaf and its molecular weight was found to be 19kDa. Recognition of the molecular factor responsible for bactericidal activity in herbs will pave the way to commercialize it for disease management.

INTRODUCTION: Infectious disease can become a threat to public health in this world. The use of medicinal plants for the treatment of various diseases is an old practice in most countries and it still offers an enormous potential source of new anti-infective agents. Inhibition of microbial activity using plant extracts has gained an importance due to their efficacy and target specify towards pathogens [1].

Acalypha indica is an annual erect herb commonly called as “Kuppai meni”. It belongs to the family Euphorbiaceae. It is a common shrub in Indian gardens, backyards of houses and waste places through the plains of India. The root, stem and leaf of *Acalypha indica* possess herbal activity. The present study was aimed to evaluate the antibacterial potentiality of acetone, methanol and ethanol extract of *Acalypha indica* against *Staphylococcus sp, E. coli , Salmonella sp, Klebsiella pneumonai and Pseudomonas* and to analyse the protein concentration and to characterize the protein causing antibacterial activity.

MATERIALS AND METHODS:

Collection of Plant Material: Healthy leaves of *Acalypha indica were* collected from Hindusthan college field, coimbatore district. The leaves, steams and roots of *Acalypha indica* were washed thoroughly three times with water and once with distilled water. The materials were air dried in hot air oven at 55°C for 3 hours and powdered by hand crushing. The dried leaves, stem and root powdered samples were hermetically sealed in separate polythene bags until the time of the extraction.

Extraction of Plant Material: 40g of powdered leaves, stem and root was extracted successively with 200ml of acetone, ethanol and methanol in Soxhelt extractor (56-60°C) until the extract was clear. The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a sterile plastic container.

Isolation of Human Pathogens from Hospital Samples: Samples were collected from hospitalized patients in and around Coimbatore district and were plated on selective media to obtain the pathogenic isolates.

1. **Isolation of Human Pathogens:**

a. **Identification of culture:** The cultures on selective media were confirmed by gram staining method, motility test and biochemical tests.

Antimicrobial Assay:

I. **Test Organisms:** The acetone, ethanol and methanol extracts of *Acalypha indica* were tested against following pathogenic organisms isolated from patients sample*: Staphylococcus sp*, *Escherichia coli*, *Salmonella sp*, *Klebsiella pneumonia* and *Pseudomonas sp* . The cultures were subcultured and maintained on nutrient agar slants and stored at 4°C. A chemically induced precipitation reaction can be used to approximate the turbidity of a bacterial suspension. 10 test tubes or ampules of equal size and of good quality were set up. Use new tubes that have been thoroughly cleaned and rinsed. 1% chemically pure Sulphuric acid and 1.175% aqueous solution of Barium chloride was prepared. Slowly and with constant agitation, the designated amounts of two solutions were added to the tubes as shown in table to make a total volume of 10ml per tube. The tubes or ampules were sealed. The suspended Barium chloride precipitate corresponds approximately to homogenous. The Mc Farland standard tubes should be stored in the dark at room temperature. They are stable for 6 months.

II. **Inoculum Preparation:** Bacterial inoculum was prepared by inoculating a loopful of test organism in 5ml of nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbity was developed. The turbidity was matched with 0.5 Mc Farland standards [2].

III. **Determination of Antibacterial Activity:** Muller Hinton agar plates were inoculated with test organisms by spreading the bacterial inoculum on the surface of the media. Wells (8cm in diameter) were punched in the agar. Extracts with different concentrations (25µl, 50µl, 75µl and 100µl) were added into the well. Well containing solvent (acetone, ethanol and methanol) alone acts as a negative control. The plates were incubated at 37°C for 18 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (in cm).

Phytochemical Analysis

1. **Saponins:** 2g of the powered sample was boiled in 20 ml of distilled water in a water bath. 10ml of the filterate was mixed with 5 ml of distilled water shaken vigorously for a stable persistent froth. The following was mixed with 3 drops of Olive oil and shaken vigorously and then observed for the formation of emulsion.

2. **Tannis:** 0.5g of the dried powdered sample was boiled in 20 ml of water and filtered. A few drops of 0.1% Ferric chloride was added and observed for brownish or bluish black colour.

3. **Alkaloids (Meyer's Test):** 0.5g of the dried powdered sample was boiled in 20 ml of water and filtered. To a few drops of the filtrate, a drop of Meyer's reagent was added by the side of the test tube. A creamy or white precipitate indicates the test is positive.

4. **Flavonoids:** A portion of the powdered sample was heated with 10 ml ethyl acetate over a steam bath for 3 minutes Mixture was filtered. 4ml of the filtrate was shaken with 1ml diluted Ammonia. Yellow coloration indicates the presence of Flavanoid.

5. **Triterpenes:** 300mg of extract was mixed with 5 ml of Chloroform and warmed at 80°C for 30 minutes. Few drops of concentrated sulphuric acid was added and mixed well. Appearance of red colour indicates the presence of Triterpenes.

6. **Phenolic Compounds (Ferric chloride Test):** 300 mg of extract was diluted to 5ml of distilled water and filtered. To the filtrate 5% Ferric chloride was added. Dark green colour indicates the presence of Phenolic compounds

Characterization of active principle of the effective Plant Extracts: To characterize the active principle responsible for inhibitory action on the pathogen, protein fractions of the leaf extracts were analyzed.

Extraction of the Protein: Freshly collected leaves were cut into small bits and homogenized in a blender with chilled acetone (chilled at 20°C). The homogenate was filtered through Whatman No.1 filter paper. The sediment was washed with chilled acetone until most of the pigments were removed. The powder obtained was air dried and stored at - 20°C for further use.

Acetone powder was stirred with extraction buffer (1:20 *WN)* containing PVPP (l00mg/g of acetone powder) at 4°C for 10 min (Extraction buffer: 0.05M Tris HCI buffer, pH-7.2 containing 0.05M EDTA O.OIM b- mercaptoethanol, 25mM ascorbic acid). The slurry was centrifuged at 15,000 rpm for 20 min at 4°C. The pellet was discarded and the supernatant was retained.

Ammonium sulphate Fractionation: The supernatant was subjected to ammonium sulphate precipitations. After centrifugation at 15000 rpm for 20 min at 4°C these fractions were dissolved in minimum quantity of extraction buffer and dialysed against double distilled water using 12 kD cut off membrane at 4°C. The dialysed samples were lyophilized and and stored at -20°C.

Quantification of Protein: Protein concentration was estimated using protein-dye binding method [3].

Protein separation on SDS-PAGE and Staining Procedure: Proteins were subjected to electrophoresis on sodium dodecyl sulphate (SDS) -polyacrylamide gel method [4].

Sample preparation and Loading: The samples were processed as follows; 300mg of ammonium sulphate fractions were taken in eppendorf tube. The total volume was made upto 25 ml with the gel-loading buffer. The tubes were vortexed thoroughly and incubated at 42°C for 1h in dry bath.

After incubation the samples were again vortexed thoroughly and centrifuged at 10,000 rpm for 10 min at room temperature to remove debris if any and the supernatant was loaded onto the gel. The protein bands in the gels were visualized by using CBB stain. The gels were destained using destaining solution until the bands became visible againt the clear background.

RESULTS AND DISCUSSION:

Gram staining and Motility: Gram positive (1 strain) and gram negative (4) strains were identified from the samples (**table 1**).

TABLE 1: GRAM STAINING

S. No.	ORGANISMS	SELECTIVE MEDIA	GRAM STAINING	MOTILITY
1	*E. coli*	Mac conkey agar	Gram negative, rod	Motile
2	*K.pneumoniae*	Mac conkey agar	Gram negative, rod	Non motile
3	*Pseudomonas sp*	Cetrimide agar	Gram negative, rod	Motile
4	*Salmonella sp*	Salmonella Shigella agar	Gram negative, rod	Motile
5	*Staphylococcus sp*	Blood agar	Gram positive, cocci in clusters	Non motile

Biochemical methods: (Tables 2 and 3)

TABLE 2: BIOCHEMICAL METHODS

Organism	Catalase	Oxidase	Coagulase
Staphylococcus sp	Positive	Negative	Positive

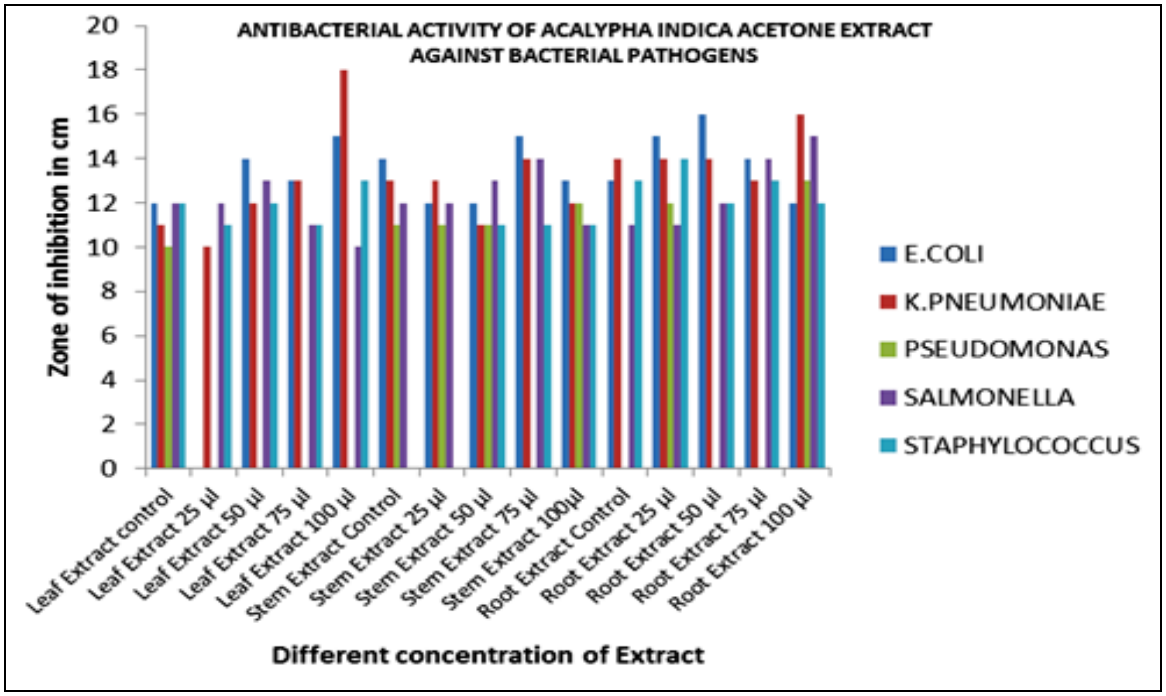
TABLE 3: BIOCHEMICAL METHODS

S. No.	Organism	Indole	MR	VP	Citrate
1	*E. coli*	+	+	-	-
2	*Pseudomonas*	+	+	-	+
3	*K. pneumoniae*	-	-	+	+
4	*Salmonella*	+	+	-	+

Antimicrobial Assay: (Table 4, graph 1)

TABLE 4: ANTIBACTERIAL ACTIVITY OF *ACALYPHA INDICA* ACETONE EXTRACT AGAINST BACTERIAL PATHOGENS

S. No.	ORGANISMS	Concentration of extract (Zone in cm)														
		LEAF EXTRACT					STEM EXTRACT					ROOT EXTRACT				
		C	25	50	75	100	C	25	50	75	100	C	25	50	75	100
1	*E.coli*	12	-	14	13	15	14	12	12	**15**	13	13	15	**16**	14	12
2	*K.pneumoniae*	11	10	12	13	**18**	13	13	11	14	12	14	14	14	13	**16**
3	*Pseudomonas*	10	-	-	-	-	11	11	11	-	12	-	12	-	-	13
4	*Salmonella*	12	12	13	11	10	12	12	13	14	11	11	11	12	14	15
5	*Staphylococcus*	12	11	12	11	13	-	-	11	11	11	13	14	12	13	12



GRAPH 1: ANTIBACTERIAL ACTIVITY OF *ACALYPHA INDICA* ETHANOL EXTRACT AGAINST BACTERIAL PATHOGENS

Antibacterial activities of acetone, methanol and ethanol and extracts of *Acalypha indica* was assayed against various bacterial pathogens. The acetone leaf extract of *Acalypha indica* showed maximum inhibition zone to *K. pneumoniae* (18 cm). *Pseudomonas* showed no zone which indicates its resistance towards the extract. The acetone stem extract of *Acalypha indica* showed maximum inhibition zone to *E. coli* (15 cm). The acetone root extract of *Acalypha indica* showed maximum inhibition zone to *K. pneumoniae* (16 cm) and *E. coli* (16 cm).

The ethanol leaf extract of *Acalypha indica* showed maximum inhibition zone to *E. coli* (22cm). The ethanol stem extract of *Acalypha indica* showed maximum inhibition zone to *Staphylococcus* (18 cm). The ethanol root extract of *Acalypha indica* showed maximum inhibition zone to *Salmonella* (17 cm) (**table 5, graph 2**).

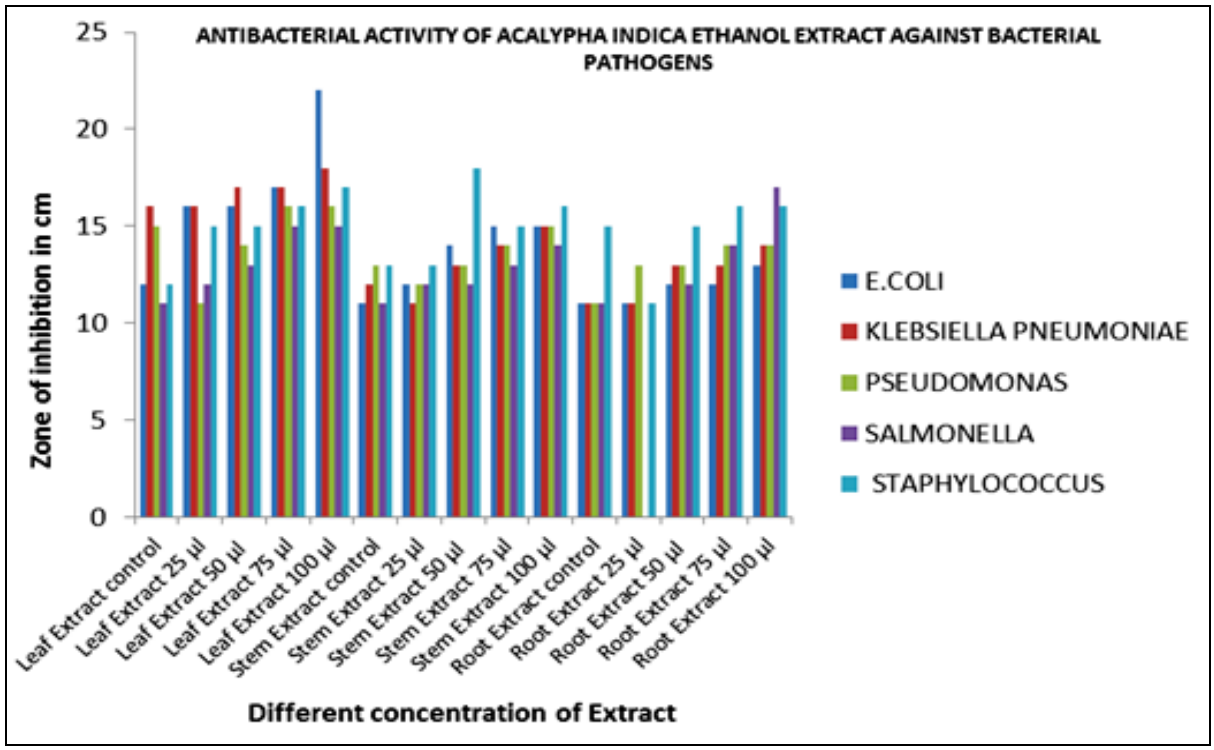
The methanol leaf extract of *Acalypha indica* showed maximum inhibition zone to *K. pneumoniae* (20 cm). The methanol stem extract of *Acalypha indica* showed maximum inhibition zone to *Salmonella (*18 cm*).* The methanol root extract of *Acalypha indica* showed maximum inhibition zone to *Pseudomonas* and *Salmonella* (19 cm) **table 6, graph 3)**.

The antibacterial activity of some Indian medicinal plants were evaluated [5]. The extract of *Acalypha indica* was tested against different bacterial pathogens. The extract of *Acalypha indica* showed 9 cm inhibition zone to *Escherichia coli* and no zone was showed against *Staphylococcus aureus*, *Salmonella typhi* and *Shigella flexneri*. Alcoholic extract of *Acalypha indica* showed 10 cm inhibition zone towards *Staphylococcus aureus* and *Salmonella typhi*.

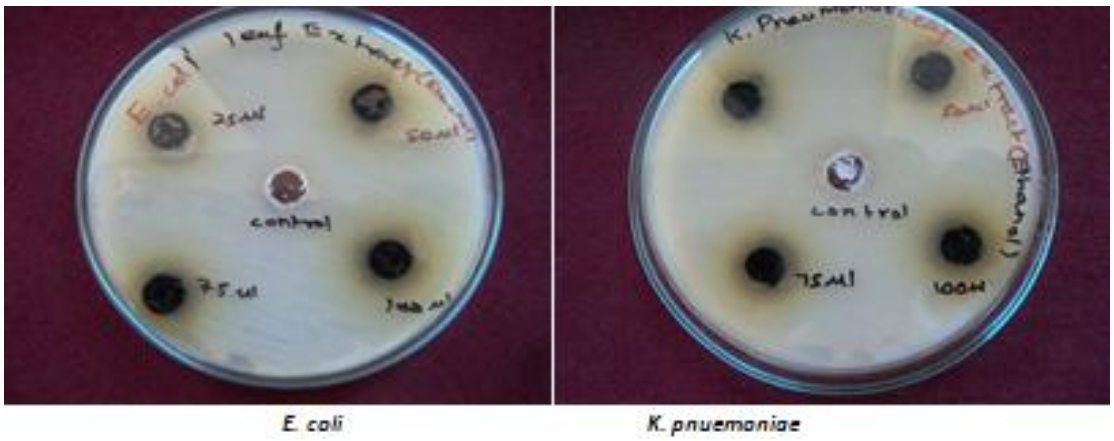
In this study ethanol was best solution for extracting the effective anti microbial substances from the medicinal plant *Acalypha indica* than acetone and methanol. The ethanol extract of *Acalypha indica* showed effective results against most of test organisms but the acetone extract of *Acalypha indica* was low effective against all the microorganisms. Some studies concerning the effectiveness of extraction methods highlight that methanol extract yields higher antibacterial activity than n-hexane and ethyl acetate[6].Whereas other report that chloroform is better than methanol and benzene [7]. It is clear that using organic solvents provides a higher efficiency in extracting compounds for antimicrobial activities compared to water based method [8].

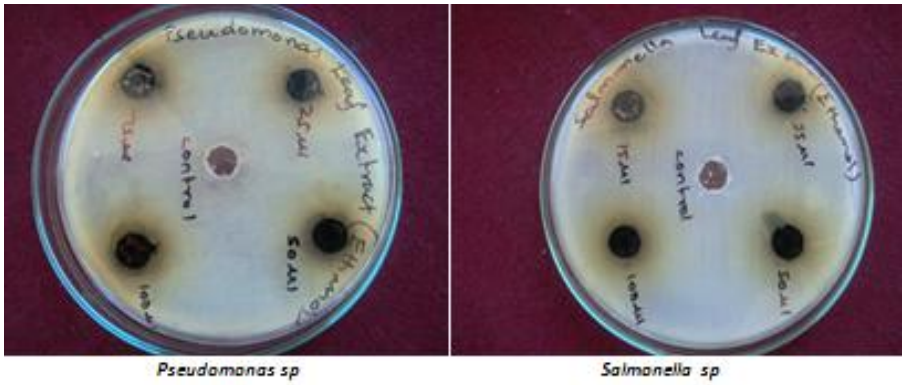
TABLE 5: ANTIBACTERIAL ACTIVITY OF *ACALYPHA INDICA* ETHANOL EXTRACT AGAINST BACTERIAL PATHOGENS

S. NO.	ORGANISMS	Concentration of extract (Zone in cm)														
		LEAF EXTRACT					STEM EXTRACT					ROOT EXTRACT				
		C	25	50	75	100	C	25	50	75	100	C	25	50	75	100
1	*E.coli*	12	16	16	17	**22**	11	12	14	15	15	11	11	12	12	13
2	*K.pneumoniae*	16	16	17	17	18	12	11	13	14	15	11	11	13	13	14
3	*Pseudomonas*	15	11	14	16	16	13	12	13	14	15	11	13	13	14	14
4	*Salmonella*	11	12	13	15	15	11	12	12	13	14	11	-	12	14	**17**
5	*Staphylococcus*	12	15	15	16	17	13	13	**18**	15	16	15	11	15	16	16

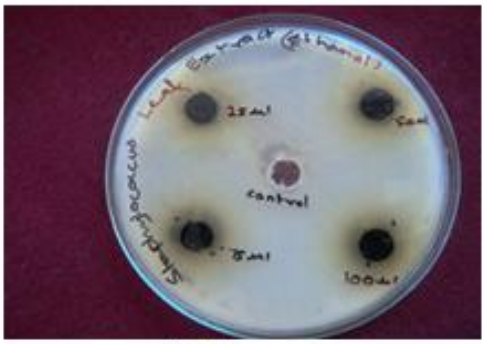


GRAPH 2: ANTIBACTERIAL ACTIVITY OF *ACALYPHA INDICA* ETHANOL EXTRACT AGAINST BACTERIAL PATHOGENS





Pseudomonas sp *Salmonella sp*

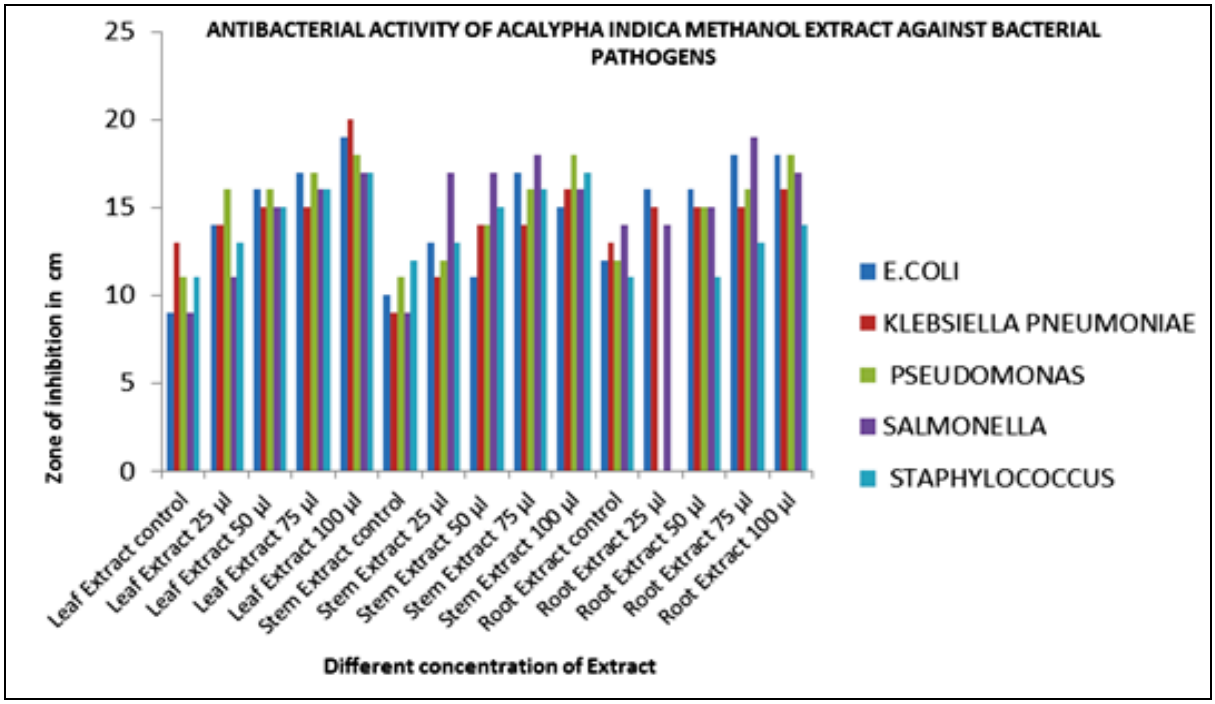


Staphylococcus sp

ANTIBACTERIAL ACTIVITY OF *ACALYPHA INDICA* ETHANOL LEAF EXTRACT

TABLE 6: ANTIBACTERIAL ACTIVITY OF *ACALYPHA INDICA* METHANOL EXTRACT AGAINST BACTERIAL PATHOGENS

S. NO.	ORGANISMS	Concentration of extract (Zone in cm)														
		LEAF EXTRACT					STEM EXTRACT					ROOT EXTRACT				
		C	25	50	75	100	C	25	50	75	100	C	25	50	75	100
1	*E.coli*	09	14	16	17	19	10	13	11	17	15	12	16	16	18	18
2	*K.pneumoniae*	13	14	15	15	**20**	09	11	14	14	16	13	15	15	15	16
3	*Pseudomonas*	11	16	16	17	18	11	12	14	16	**18**	12	-	15	16	18
4	*Salmonella*	09	11	15	16	17	09	17	17	**18**	16	14	14	15	**19**	17
5	*Staphylococcus*	11	13	15	16	17	12	13	15	16	17	11	-	11	13	14



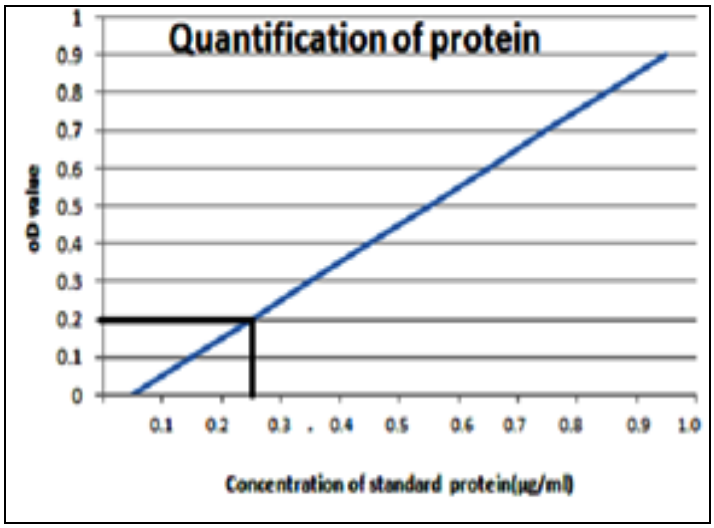
GRAPH 3: ANTIBACTERIAL ACTIVITY OF *ACALYPHA INDICA* METHANOL EXTRACT AGAINST BACTERIAL PATHOGENS

Qualitative analysis of *Acalypha indica* leaf: Phytochemical analysis of *Acalypha indica* extract showed the presence of Flavonoids, Triterpenoids and Phenolic compounds. Antibacterial activity of *Acalypha indica* was due to the presence of Flavonoids, Triterpenoids and Phenolic compounds (**table 7**).

TABLE 7: RESULTS OF PHYTOCHEMICAL TESTS

S. NO.	Phytochemical tests	Results
1	Sapononins	Absent
2	Tannins	Absent
3	Alkaloids	Absent
4	Flavonoids	Present
5	Triterpenoids	Present
6	Phenolic compounds	Present

Quantification of protein: Protein concentration in *A. indica* leaf was estimated as 0.25 µg/ml (**Graph 4**)

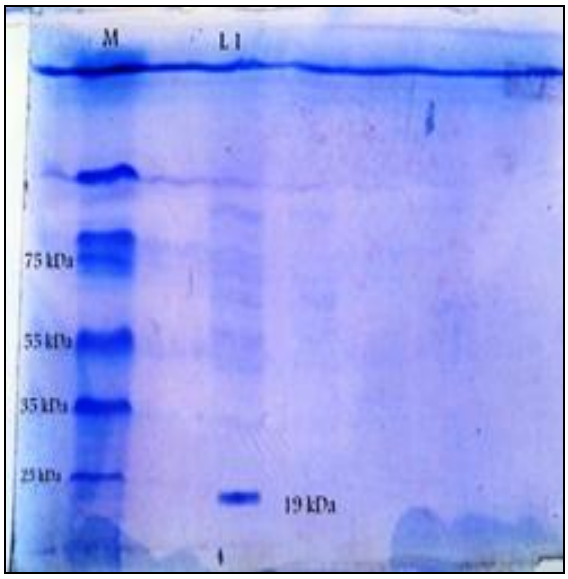


GRAPH 4: QUANTIFICATION OF PROTEIN

Protein separation on SDS-PAGE: The molecular weight of the inhibitory protein was determined by the electrophoretic analysis of purified ammonium sulphate fractions. In *A. indica,* protein fraction was found to be 19kDa.

In the present study the antimicrobial factors of *A. indica* were found to be proteinaceous. Many workers also reported the proteinaceous nature of anti-microbial factor. *A. indica* possessed thermolabile antimicrobial factors that reduced the growth of human pathogenic bacteria.

SDS PAGE Protein pattern of effective *Acalypha indica* leaf:



M- Low molecular weight standard; L1- Acalypha leaf protein fraction

CONCLUSION: The study of antibacterial activity of herbal plant extract of *Acalypha indica* showed that the ethanol leaf extract shows promising antibacterial activity against bacterial human pathogens when compared to acetone and methanol extract. The results also indicated that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results. Antibacterial activity of *Acalypha indica* was due to the presence of Flavonoids, Triterpenoids, Phenolic compounds and inhibitory protein whose molecular weight was found to be 19kDa.

These plants could serve as useful source of new antimicrobial agents. *A.indica* possessed thermolabile antimicrobial factors that reduced the growth of human pathogenic bacteria. Various pathogenic bacteria have developed resistance to many of the currently available antibiotics. The root, stem and leaf of *Acalypha indica* possess Antibacterial activity against human pathogens.

Historically, plants have provided a good source of anti-infective agents and many of them remain highly effective in the fight against microbial infections. Besides, they are cost- effective and have fewer side effects [9].

REFERENCES:

1. Renganathan S, Rajesh Kanna C, Baskar G: Studies on antibacterial activity of *Leucas aspera* extracts. Research Journal of Biological Science. 2009; 1(4): 23.
2. Chakraborthy *et al*: Antimicrobial effects of chitosans and chitooligosaccharides, upon *Staphylococcus aureus* and *Escherichia coli* in food model systems**.** Food Microbiology. Volume 25, Issue 7, October 2008; 922–928.
3. Bradford MM: Anal. Biochem. 1976; 72: 248-254.
4. Laecmli UK, Molbert E, Showe M and Kellenberger E: J. Mol. Biol. 1970; 49, 99.
5. Sumathi G and Pushpa B: Evaluation of antibacterial activity of some Indian medicinal plants. Asian Journal of Microbiology, Biotechnology and Environmental Sci. 2007; 9: 201- 205.
6. Sastry VMVS and Rao GRK: Antibacterial extraction using benzene, chloroform and methanol. Botanica Marina. 1994; 37: 357-360.
7. Febles CI, Arias A, Gil-Rodriguez MC, Hardisson A, SierraLopez A: *In vitro* study of antimicrobial activity in algae (Chlorophyta, Phaeophyta and Rhodophyta) collected from the coast of Tenerife (in Spanish). Anuario del Instituto de Estudios Canarios. 1995; 34: 181-192.
8. Lima-Filo JVM, Carvola AFFU and Freitas SM: Antibacterial activity of extract of six macro algae from the North eastern Brazilian Coast. Brazilian Journal of Microbiology. 2002; 33: 311-313.
9. Reminton S: Pharmaceutical Sciences. 17th ed. Mack Publishing Co. Eastan. 1991.

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