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EVALUATION OF ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL PLANTS ON CERTAIN HUMAN BACTERIAL PATHOGENS

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ABSTRACT In view of harmful effects caused by synthetic antibiotics in treating bacterial infections in human beings many studies were carried out to find some alternative methods using safe and natural plant products. In the present study Methanolic extracts of twenty plant species were evaluated for their anti microbial activity against twelve selected bacterial human pathogens by well diffusion method. out of twenty plant extracts evaluated six of them namely *Tribulus terrestris , Sphaeranthus indicus, Euphorbia hirta, Hyptis saveolens Tagetes patula*, have shown significant anti bacterial activity against eight tested pathogens which are *Staphylococcus aureus, Pseudomonas aurigenosa, Streptococus pneumoniae, Eschericia coli, Streptococcus pyogenes, Klebsiella pneumonia, Citrobacter koseri.*

KEYWORDS : Antimicrobial activity, Methanolic plant extracts, Bacterial infections in humans.

Introduction:

Health is considered as a blessing . an orderly and healthy life style any disturbances, damages or interruptions to the perfectly orderly function of human body were caused by human organisms. Man from the ancient days have gained the knowledge of using plant products in various forms and variety of ways imbibing them into their regular life style to master the art of controlling diseases caused by micro organisms was in practice in every known civilization in human history. With the development of modern medicine, the use of synthetic antibiotics to combat almost all kinds of bacterial infections and the disease caused by them in humans has been increased rapidly. But the harmful effects of these synthetic drugs on the health of the individuals using them has pushed medical scientist to search for much safer and more convenient reliable alternatives instead of the present commercially available synthetic antibiotics.

Materials and methods:

Collection of plant materials: Twenty different plants were selected from literature and through field observation. Plants were collected from the Visakhapatnam and vizianagaram district, Andhra Pradesh India. Whole plants were screened for their anti bacterial activity. The collected material was washed thoroughly with distilled water and then the material are shade dried on the sterile blotter (M.K. Khoka 2012)to a constant weight for a period of 45 days. The collected plant specimens were identified with herbarium available in the department of Botany, Andhra University, Visakhapatnam. (table 1)

Solvent extraction of plant material:

The completely shade dried plant material was ground into a coarsely powder using electric blender. Dried powder was subjected to soxhlet extraction using methanol as a solvent. The whole material was then subjected to distillation at 62^0c about eight hours to remove the solvent. After distillation different extracts obtained were concentrated with rotary evaporator and brought to complete dryness over a water bath to yield the crude extracts. These extracts were collected, labelled and stored at 4^0c for further use.

Collection of Microbial cultures: Based on common diseases in Humanbeings twelve pathogenic bacteria were selected to perform the anti bacterial action of test samples all the cultures were collected from TRIMS VISAKHAPATNAM.

In-vitro anti microbial assay: The crude extracts of different plants were subjected to anti microbial assay using well plate method(Murray et al., 1995). For the bioassay studies, the media used is Mullar Hinton Agar. By avoiding any significant mixing the culture is good for inoculating microbes on surface of the medium as required for isolation of pure cultures.

Preparation of culture: A loop full of clinically tested pure culture was reconstituted in sterile peptone water to produce a suspension of microbial cells

Preparation of plates for Agar diffusion method: To prepare media, it requires twenty plates of Muller Hinton agar for each organism. 500ml of distilled water 19.5 grms of MH agar was weighted and dissolved in a conical flask. Then it was autoclaved at 15lbs pressure at 121^0c for 20 mins. After sterilization, media was aseptically distributed into Petri plates and allowed to solidify. The assay was performed by using well plate method. To determine the potential of plant extract there were diluted up to 500mg/ml, 250mg/ml,125mg/ml,62.5mg/ml of dimethyl sulfoxide solution. from each dilution twenty µl was introduced into four wells and allowed to diffuse for 45mins. The plates were incubated at 37^0c for 24 hours.

Results:

A total of twenty methanolic extracts belonging to different plant species were used in screening in vitro antibacterial study. All the screened plant extracts exhibited activity against at least one microorganism. among the twenty methanolic plant extracts six extracts showed significant antibacterial activity against eight tested pathogens based on zone of inhibition. These plant species are *Tribulus terrestris, Sphaeranthus indicus, Euphorbia hirta, Tagetes patula, Phyllanthus madras patensis and hyptis suaveolens.*

Among the six plants extracts that shown significant anti bacterial activity *Tribulus terrestris* Has given highest inhibition zone with 22mm diameter against *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumonia*, and with 20mm diameter of inhibition zone for Bacillus subtilis, and Ecoli. This extract have shown significant activity against *Citrobacter koseri,Streptococcus pyogenes, enterobacter aerogenes* with 12mm,6mm diameter of inhibition zone respectively at 125mg/ml concentration.(table 2 Fig 1)

Sphaeranthus indicus plant has given highest zone of inhibition of 16mm diameter against *Streptococcus pneumonia, Bacillus subtilis, Streptococcus pyogenes, and klebsiella pneumonia.(table 3Fig 2)*

Phyllanthus madras patensis extract has also exhibited significant activity against 6 pathogens with 18mm,14mm,112mm,6mmdiameter of inhibition zone against *Staphylococcus aureus,* ecoli, Streptococcus pneumonia, bacillus subtilis, Pseudomonas aeruginosa respectively. (table 4 Fig 3)

Euphorbia hirta has given its highest inhibition against *Staphylococcus aureus, Klebsiella pneumonia,* with 16mm diameter of inhibition zone and with 14mm, 12mm inhibition zones against

Ecoli, streptococcus pneumonia, bacillus subtilis,respectively at 125 mg/ml concetraction.(table 5 Fig 4)

Tagetes patula has given significant inhibition against streptococcus pneumonia and *Staphylococcus aureus* with 12mm diameter of inhibition zone each and 10mm diameter od inhibition zone against ecoli,(table 6 Fig 5)

Table 1:List of plants and collection area:

S. No.	Name of the Plant	Place of collection
1	*Tribulus terrestris Linn*	Lakkidam, Vizianagaram dist.
2	*Sphaeranthus indicus Linn*	Vizianagaram
3	*Tagetes patula Linn*	Vizianagaram
4	*Phyllanthus madraspatensis Linn*	Lakkidam, Vizianagaram dist.
5	*Hyptis suaveolens (L.) Poit*	Gajuwaka
6	*Euphorbia hirta Linn*	Visakhapatnam
7	*Ocimmum tenuiefloram Linn*	Gajuwaka
8	*Phyllanthus neruri Linn*	Visakhapatnam
9	*Cromolaena odorata Linn*	Andhra University Campus
10	*Fiora vitifolia (L.) Mattei*	Andhra University Campus
11	*Blumea mollis DC.*	Vizianagaram
12	*Acalypha indica Linn*	Vizianagaram
13	*Abutilon indicum Linn*	Andhra University Campus
14	*Wrightia tinctoria Roxb.*	Andhra University Campus
15	*Portulaka oleracea Linn*	Visakhapatnam
16	*Croton bonplandianum Linn*	Visakhapatnam
17	*Eclipta alba Linn*	Lakkidam, Vizianagaram dist.
18	*Coriandrum sativum Linn*	Vizianagaram
19	*Centella aciatica Linn*	Vizianagaram
20	*Heliotropium indicum Linn*	Gajuwaka

Tables 2: **Antimicrobial activity of *Tribulus terrestris* Linn**

Name of the Pathogen	Conc. of extract in mg/ml				Activity
	500	250	125	62.5	
Streptococcus pneumoniae	34	24	22	18	High
Pseudomonas aeruginosa	32	28	22	18	High
Staphylococcus aureus	28	24	22	20	High
Bacillus subtilis	28	24	20	16	High
Escherichia coli	28	22	20	16	High
Citrobacterkoseri	22	18	12	08	Moderate
Streptococcus pyogenes	14	12	06	02	Low
Enterobacter aerogenes	12	08	06	02	Low
Klebsiella pneumoniae	06	nil	nil	nil	Nil
Serratia marcescens	04	nil	nil	nil	Nil
Proteus vulgaris	Nil	nil	nil	nil	Nil
Shigella dysenteriae	Nil	nil	nil	nil	Nil

Activity of plant extract at a concentration of 125mg/ml-
Low – 6-9, Moderate – 10-15, High – more than 15
Volume of plant extract taken - 20µL.
Diameter of Zone of Inhibition is indicated in mm.

Table 3: Antimicrobial activity of *Sphaeranthus indicus* Linn.

Name of the Pathogen	Conc. of extract in mg/ml				Activity
	500	250	125	62.5	
Staphylococcus aureus	24	14	10	08	Moderate
Streptococcus pneumoniae	22	20	16	14	High
Streptococcus pyogenes	22	20	16	12	High
Klebsiella pneumoniae	22	20	16	10	High
Bacillus subtilis	20	18	16	12	High
Pseudomonas aeruginosa	20	18	14	10	Moderate
Enterobacter aerogenes	20	18	12	nil	Moderate
Escherichia coli	18	12	10	06	Moderate
Serratia marcescens	08	04	02	nil	Nil
Proteus vulgaris	02	nil	nil	nil	Nil
Citrobacter koseri	10	08	06	04	Low
Shigella dysenteriae	Nil	nil	nil	nil	Nil

Activity of plant extract at a concentration of 125mg/ml-
Low – 6-9, Moderate – 10-15, High – more than 15
Volume of plant extract taken - 20µL.
Diameter of Zone of Inhibition is indicated in mm.

Table 4: Antimicrobial activity of *Phyllanthus madraspatensis* Linn.

Name of the Pathogen	Conc. of extract in mg/ml				Activity
	500	250	125	62.5	
Staphylococcus aureus	28	20	18	04	High
Klebsiella pneumoniae	22	18	14	06	High
Bacillus subtilis	22	16	12	06	Moderate
Escherichia coli	20	18	14	10	Moderate
Streptococcus pneumoniae	18	16	12	04	Moderate
Pseudomonas aeruginosa	12	10	06	04	Low
Serratia marcescens	04	02	nil	nil	Low
Proteus vulgaris	02	nil	nil	nil	Low
Enterobacter aerogenes	Nil	nil	nil	nil	Nil
Citrobacter koseri	Nil	nil	nil	Nil	Nil
Streptococcus pyogenes	Nil	nil	nil	Nil	Nil
Shigella dysenteriae	Nil	nil	nil	Nil	Nil

Activity of plant extract at a concentration of 125mg/ml-
Low – 6-9, Moderate – 10-15, High – more than 15
Volume of plant extract taken - 20µL.
Diameter of Zone of Inhibition is indicated in mm.

Table 5: Antimicrobial activity of *Euphorbia hirta* Linn.

Name of the Pathogen	Conc. of extract in mg/ml				Activity
	500	250	125	62.5	
Staphylococcus aureus	24	20	16	14	High
Klebsiella pneumoniae	24	18	16	12	High
Escherichia coli	24	20	14	10	Moderate
Streptococcus pneumoniae	24	16	12	08	Moderate
Bacillus subtilis	24	16	12	08	Moderate
Pseudomonas aeruginosa	12	10	08	04	Low
Streptococcus pyogenes	08	06	02	nil	Low
Enterobacter aerogenes	06	02	02	nil	Low
Proteus vulgaris	04	02	nil	nil	Nil
Serratia marcescens	02	nil	nil	nil	Nil
Citrobacter koseri	02	nil	nil	nil	Nil
Shigella dysenteriae	Nil	nil	nil	nil	Nil

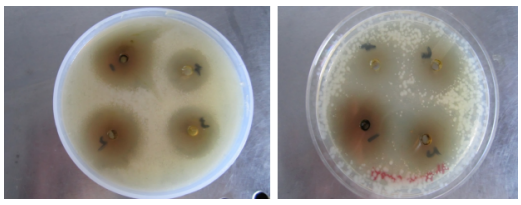
Activity of plant extract at a concentration of 125mg/ml-
Low – 6-9, Moderate – 10-15, High – more than 15
Volume of plant extract taken - 20µL.
Diameter of Zone of Inhibition is indicated in mm.

Table 6:Antimicrobial activity of *Tagetes patula* Linn.

Name of the Pathogen	Conc. of extract in mg/ml				Activity
	500	250	125	62.5	
Staphylococcus aureus	20	16	12	08	Moderate
Pseudomonas aeruginosa	20	14	10	06	Moderate
Streptococcus pneumoniae	18	14	12	08	Moderate
Escherichia coli	18	14	10	06	Moderate
Streptococcus pyogenes	18	14	08	04	Low
Bacillus subtilis	12	10	06	02	Low
Citrobacter koseri	10	08	06	04	Low
Proteus vulgaris	10	08	06	02	Low
Klebsiella pneumoniae	06	nil	nil	nil	Nil
Serratia marcescens	02	nil	nil	nil	Nil
Enterobacter aerogenes	Nil	nil	nil	nil	Nil
Shigella dysenteriae	Nil	nil	nil	nil	Nil

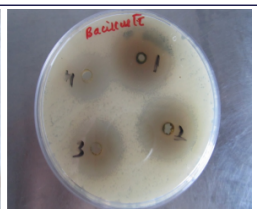
Activity of plant extract at a concentration of 125mg/ml-
Low – 6-9, Moderate – 10-15, High – more than 15
Volume of plant extract taken - 20µL.
Diameter of Zone of Inhibition is indicated in mm.

Antimicrobial Activity of *Tribulus terrestris Fig 1*



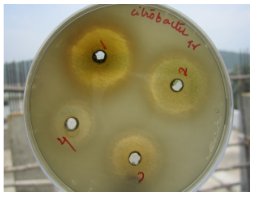
Streptococcus pneumoniae ***Pseudomonasaurginosa***

Staphylococcus aureus



Bacillus subtilis

Escherichia coli

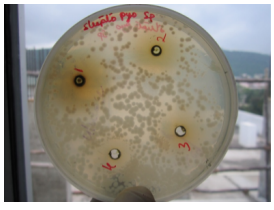


Citrobacter koseri

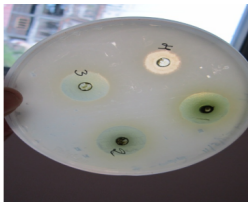
Antimicrobial Activity of *Sphaeranthus indicus Fig 2*

Staphylococcus aureus

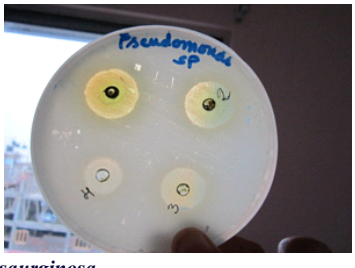
Streptococcus pneumoniae



Staphylococcus pyogenes

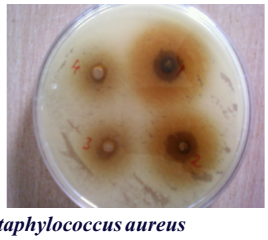


Klebsiella pneumoniae

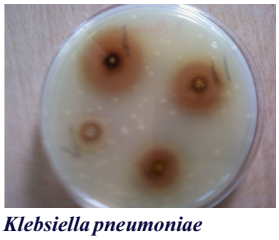


Pseudomonasaurginosa

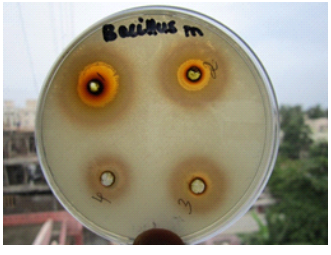
Antimicrobial Activity of *Phyllanthus madraspatensis Fig 3*



Staphylococcus aureus

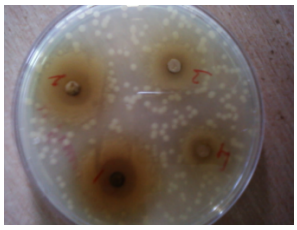


Klebsiella pneumoniae

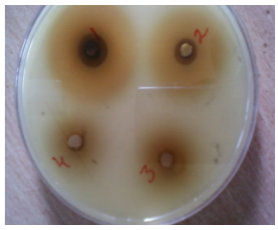


Bacillus subtilis

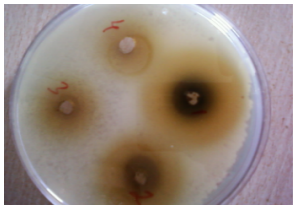
Antimicrobial Activity of *Euphorbia hirta Fig 4*



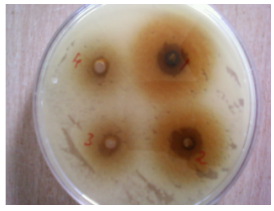
Staphylococcus aureus



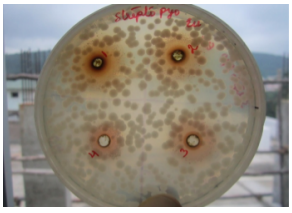
Klebsiella pneumoniae



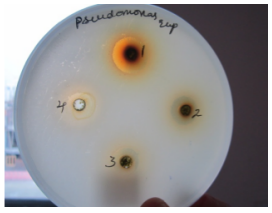
Escherichia coli



Bacillus subtilis

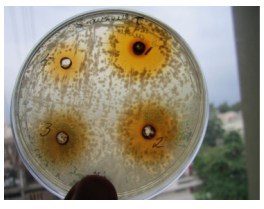


Streptococcus pneumoniae



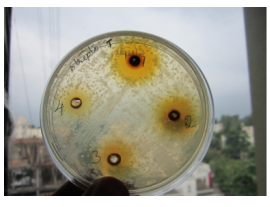
Pseudomonasaurginosa

Antimicrobial Activity of *Tagetes patula Fig 5*

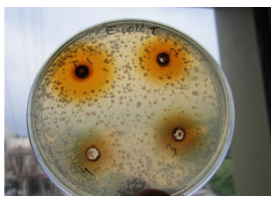


Staphylococcus aureus

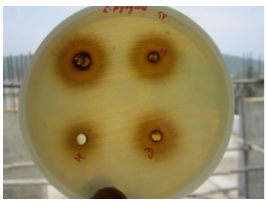
Pseudomonas aurginosa



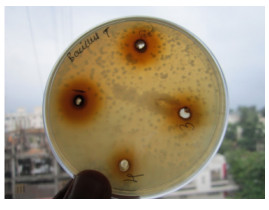
Streptococcus pneumoniae



Escherichia coli



Streptococcus pyogenes



Bacillus subtilis

In the above figures, 1, 2, 3 and 4 indicate loaded concentrations of 500 mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml, respectively, of 20 µl of methanolic extracts dissolved in DMSO solution.

Discussion : Infectious diseases are major cause of mortality world wide .there has been increasing incidence of multiple resistances in human pathogenic micro organisms in recent years, largely due to the indiscriminate use of commercial anti microbial drugs commonly employed in the treatment of infectious diseases. This situation provide the force to the search for new antimicrobial substances from various source like medicinal plants. the plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made contributions to human health and well being. the

use of plant extracts with known anti microbial properties can be of great significance for therapeutic treatment.

In the present study of preliminary screening of anti bacterial activity exhibited significant activity against twelve pathogenic bacterial strains. *Tribulus terrestris* showed maximum inhibitory zone against human pathogens *Streptococcus pneumonia*, *Staphylococcus aureus, Pseudomonas aerogenosa*.

The Methanolic extract of *Heliotropium indicum, Centella asiatica*, and *Croton bonplandianum* have shown least inhibitory activity.

The anti microbial assay by agar well diffusion method revealed that methanolic extract of medicinal plants showed maximum activity against the tested isolates.

Results obtained from this study indicated that methanolic extract of *Tribulus teresstris Sphaeranthus indicus*, *Euphorbia hirta,* and *Tagetes patula* have proved to be more effectively inhibiting the bacterial pathogen than the broad spectrum antibiotics like Rifampicin and Ciprofloxacin. The nature and number of antibacterial compounds involved in each extract of present study is not fully discovered, the broad spectra of the activity of these chosen plant extracts were promising. The present investigation collaborates with the study of Ahmed et al.,(2009), Varsha et al., (2010), Upadhya et al., (2010), Sesikala et al., (2014).

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