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RESEARCH ARTICLE

In vitro evaluation of antibacterial activity of leaf extracts

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SUMMARY

Leaves of *Lantana camara* L.,*Clematis gouriana* Roxb.,*Tridex procumbens* L., *Tephrosia purpurea* Pers. and *Piper betle* L. extracted in aqueous and ethanol medium were evaluated for their effect on the growth of *Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Salmonella typhimurium*. Extract of *C. gouriana* was found to have no antibacterial effect. The aqueous and ethanol extract of only *L.camara* could show the inhibitory activity on bacteria. Ethanol extract was more effective than aqueous extract. *P. aeruginosa* and *S. typhimurium* was found to be resistant to all plant extracts except *P.betle.* There was no effect of *P. betle* extract on *E.coli.*

Key Words **:** Leaf extracts, Bacteria, Effect, Inhibition

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Plants represent a rich source for biologically active compounds having potential to inhibit the growth of multi -drug resistant bacteria. A number of higher plants as source for new drugs are still largely unexplored. Only a small percentage of plant species has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. The major classes of plant compounds that exhibit antimicrobial activity are phenolics,terpenoids and essential oils, alkaloids, lectines and polypeptides and polyacelytenes (Cowan,1999).

Recently, exploitation of wild plants for medicinal purposes has gained more acceptances in many countries of the world. The antimicrobial agents in plants have enormous therapeutic potential and can form the base for the development of new medicines (Trease and Evans, 1972). All plant parts are good source of antibacterial compounds (Dorman and Deans, 2000). Goyal *et al.* (2008) reported greater antibacterial activity in leaves of *Catharanthus roseus* than in other parts of plant.

A number of workers (Naqvi *et al.,* 1991; Gislene *et al.,* 2000; Srivastava and Bohra, 2005; Nair and Chandra, 2007; Nair *et al.,* 2005; Pareikh *et al.,* 2005; Negi and Sharma, 2010) have investigated the antibacterial activity of the extracts of plant parts. Indiscriminate use of synthetic antimicrobial drugs leads to the development of resistance in microorganisms. Therefore, search for alternative drugs from natural sources is needed to counter the resistant microorganisms.

Thus, in the present investigation, leaves of five plants *viz., Lantana camara* L., *Clematis gouriana* Roxb.,*Tridex procumbens* L., *Tephrosia purpurea* Pers. and *Piper betle* L. were extracted in water and ethanol and tested *in vitro* for their antibacterial effect on one gram positive bacterium *Staphylococcus aureus* and four gram negative bacteria *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhimurium* .

MATERIALS AND METHODS

The leaves of the plants collected from the Kalsubai regions of Western Ghats were washed under running tap water and surface sterilized by 0.1% w/v $HgCl_2$, followed by rinsing twice in distilled water so as to remove the traces of $HgCl_2$. These leaves were then dried in shade at room temperature, homogenized to fine powder and stored in airtight bottles.

MEMBERS OF THE RESEARCH FORUM

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Aqueous extraction:

Ten g of dry leaf powder of each plant was extracted in 100 ml of distilled water for 6 hrs on hot water bath. After every 2hrs, it was filtered through eight layers of muslin cloth and centrifuged at 5000 r pm for 15min.The supernatant was collected and condensed in boiling water bath until the water was evaporated and the extract thus obtained was stored in brown bottles at 4^0C for further experiments.

Solvent extraction :

Ten g of dry leaf powder of each plant was extracted in 50 ml of ethanol on rotary shaker at 150 rpm for 24 hrs.Therafter, it was filtered through eight layers of muslin cloth and centrifuged at 5000 r pm for 15min. The supernatant was collected and the solvent was allowed to evaporate and the extract was stored in brown bottles at 4^0C for further experiments.

Bacterial cultures :

Five strains of bacteria *viz., Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 23564 and *Staphylococcus aureus* ATCC 25923 obtained from the National Chemical Laboratory (NCL),Pune,India were used as test microorganisms. The stock cultures were maintained on Muller Hinton Agar slants at 4^0C. The bacteria were revived on sterilized MHA medium in Petri plates at 37^0C.

Inoculum preparation :

Bacterial strains were grown to exponential phase in saline medium (0.85% NaCl) at 37^0C for 24hrs and adjusted to final density of 10^4cfu / ml to obtain a turbidity visually compared to 0.5 McFarland standards (Andrews, 2001).

Antibacterial activity testing :

The antibacterial activity of leaf extracts was evaluated by agar well diffusion assay (Perez *et al.,* 1990). Aqueous and ethanol leaf extracts were dissolved in distilled water and dimethyl sulfoxide (DMSO), respectively to get a concentration of 10mg / ml. 100µl inoculum (10^4cfu / ml; 0.5 MacFarland standards) of each test bacterium was spread with the help of sterile glass spreader on sterile MHA medium in Petri plates so as to achieve a confluent growth. With the help of a sterile cork borer (5 mm diameter) wells were made in the seeded agar plates. Each well was then filled with 50µl (10 mg/ml concentration) of plant extract. The plates were allowed to stand for atleast half an hour for diffusion to take place and then incubated at 37^0C for 24 hrs. DMSO and sterile distilled water was used as a negative control for the ethanolic and aqueous extracts, respectively. Different concentrations of tetracycline were tested for inhibitory effect on bacteria. The lowest concentration of 1mg / ml and 25 µg/ ml of tetracycline required to inhibit *P.aeruginosa* and other bacteria, respectively was used in positive control sets while for negative control, DMSO and distilled water was used.

RESULTS AND DISCUSSION

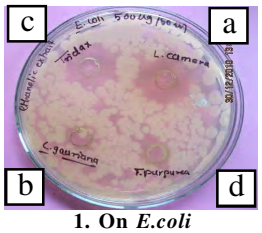
Antimicrobial activity was evaluated by measuring the diameter of zone of inhibition excluding the diameter of well against the test organism. The results are presented in Table 1.

Table 1 and Plate 1-4, reveal that the extracts of all the plants except, *C. gouriana* exhibited antibacterial activity. Ethanol and aqueous extracts of *L.camara* inhibited the growth of *E.coli, S.aureus* and *K.pneumoniae*. The ethanol extracts of *L.camara* and *T.procumbens* exhibited more antibacterial activity than other plants. Sailaja *et al.* (2010) also reported more antibacterial activity in *L.camara.*

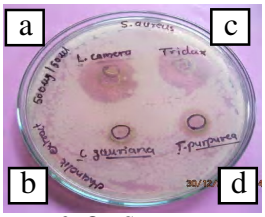
Table 1: Testing of antibacterial activity

Plant extract	Bacterial zone of inhibition (in mm)									
	E. coli		*S. aureus*		*K. pneumoniae*		*P. aeruginosa*		*S. typhimurium*	
	Aq.ext	Eth.ext	Aq.ext	Eth.ext	Aq.ext	Eth.ext	Aq.ext	Eth.ext	Aq.ext	Eth.ext
L. camara	12.33*** ± 0.47	16.67*** ± 0.47	2.00*** ± 0.00	15.33NS ± 0.47	8.33*** ± 0.94	12.67** ± 0.47	-	-	-	-
C. gouriana	-	-	-	-	-	-	-	-	-	-
T.procumbens	-	15.33*** ± 0.47	-	14.67NS ± 0.47	-	12.33** ± 0.47	-	-	-	-
T. purpurea	-	5.67** ± 0.47	-	3.00*** ± 0.82	-	2.33*** ± 0.47	-	-	-	-
P .betle	-	-	-	6.67*** ± 0.47	-	6.33*** ± 0.94	-	3.00*** ± 0.82	-	3.33*** ± 0.94
+ *ve control* Tetracycline (25 µg/ml)	7.5 ± 0.5		14.33 ± 0.94		15.33 ± 0.47		7.5 ± 0.5 #		12.66 ± 0.47	
-ve control DW / DMSO	-		-		-		-		-	

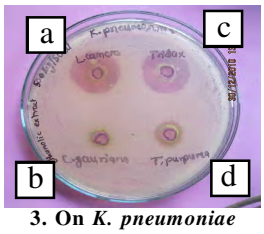
: Positive control for *P.aeruginosa* : 1 mg / ml, NS = Non -significant. ** = P < 0.01. *** = P < 0.001



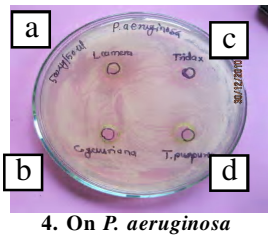
1. On *E.coli*



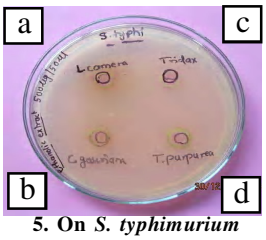
2. On *S. aureus*



3. On *K. pneumoniae*

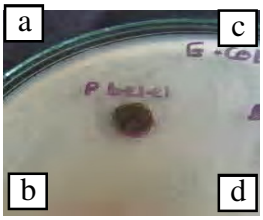


4. On *P. aeruginosa*

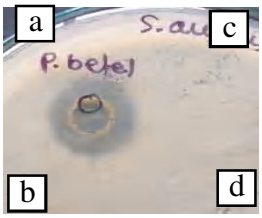


5. On *S. typhimurium*

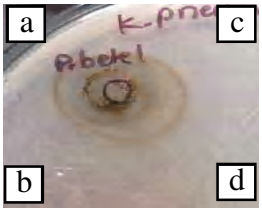
Plate 1: Zones of inhibition of bacteria due to ethanol leaf extracts of *L.camara, C.gouriana, T.procumbens, T. purpurea* (a: *L. camara,* b: *C. gouriana,* c: *T. procumbens,* d: *T. purpurea*)



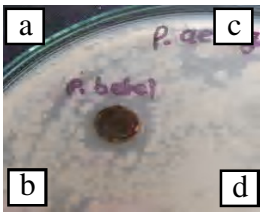
1. On *E.coli*



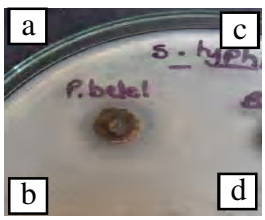
2. On *S.aureus*



3. On *K.pneumoniae*

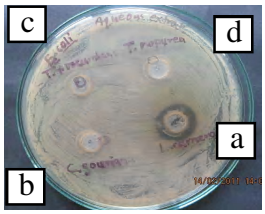


4. On *P.aeruginosa*

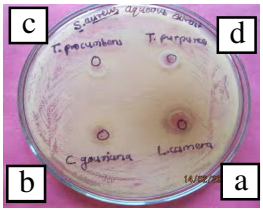


5. On *S.typhimurium*

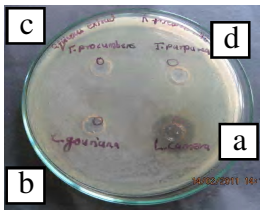
Plate 2 : Zones of inhibition of bacteria due to ethanol leaf extracts of *P. betle*



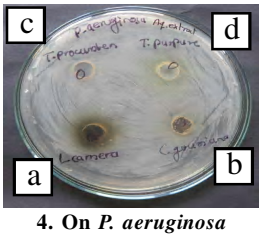
1. On *E.coli*



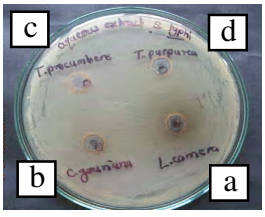
2. On *S. aureus*



3. On *K. pneumoniae*



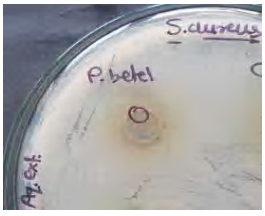
4. On *P. aeruginosa*



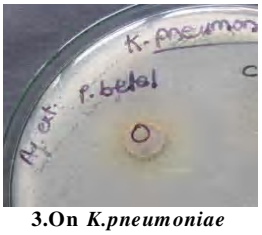
5. On *S. typhimurium*

Plate 3 : Zones of inhibition of bacteria due to aqueous leaf extracts of *L. camara, C.gouriana, T. procumbens, T. purpurea* (a: *L.camara,* b: *C. gouriana,* c: *T. procumbens,* d: *T. purpurea*).

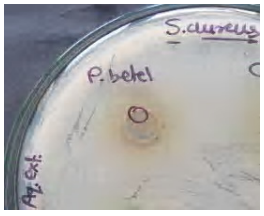
1.On *E.coli*



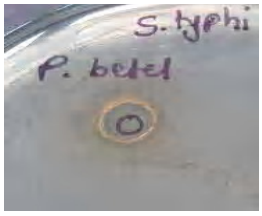
2. On *S.aureus*



3.On *K.pneumoniae*

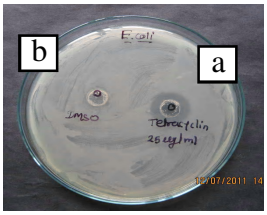


4. On *S. aureus*



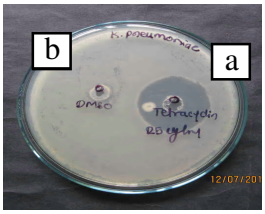
5. On *S.typhimurium*

Plate 4: Zones of inhibition of bacteria due to aqueous leaf extracts of *P.betle*

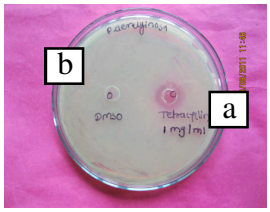


1. On *E.coli*

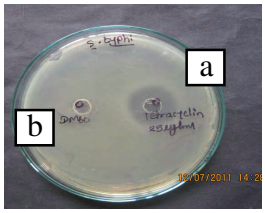
2. On *S.aureus*



3. On *K.pneumoniae*



4. On *P.aeuroginosa*



5. On *S. typhimurium*

Plate 5 : Positive control by tetracycline and negative control by DMSO (a: Tetracycline. b: DMSO)

P. aeruginosa and *S. typhimurium* did not get inhibited either by aqueous or ethanol extracts of *L. camara, T. procumbens, T. purpurea.* No inhibition zone was seen in *E.coli* treated with the extract of *P.betle*.(Plate 1 and 5). Ethanol extract showed greater antibacterial activity than aqueous extract which corroborates with the reports of Srivastava and Bohra (2005), Firas *et al.* (2008), Sailaja *et al.* (2009) and Negi and Sharma (2010). However, the ethanol extracts of *T.purpurea* and *P.betle* showed mild antibacterial activity.

Conclusion :

The metabolic compounds in plants can be a good source for the development of novel drugs to suppress the emergence of drug resistant pathogenic bacteria. Many plants need to be tested for their antibacterial properties and there is wide scope for further research in these lines.

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