Indian J. Applied & Pure Bio. Vol. 41(2), 931-936 (2026). **ISSN: 0970-2091**

Evaluation of antimicrobial activity of some higher plants against the human pathogenic Bacteria

Shreya Yadav[*], M. Jaish Beg[*] and Hemalata Yadav[**]

[*]Department of Botany, Shibli National P.G. College (MSDS University)
Azamgarh - 276001 (India)
[**]Department of Pharmacy, R.S. Pharmacy College (MSDS University)
Azamgarh - 276001 (India).
Corresponding Author E-mail. sy83338055@gmail.com
Postal address: Musepur, Sadar, Azamgarh, 276001.

Abstract

The human pathogenic bacteria *Pseudomonas aeruginosa* is a gram negative pathogen, responsible for pneumonia, skin disease, Urinary Tract Infection. They are found in intestinal tract and blood of human beings and animals, which are known to be responsible for major health problems world wide. The test pathogen *Pseu*domonas aeruginosa was collected from the sputum of patient, cultured on Mac Conkey's Agar Medium and identified by different colony characters and biochemical test. 50 % ethanolic extracts (v/v) of defferent available plant parts of 25 plants were screened for their antibacterial activity against test pathogens by 'Inhibition Zone technique ' . The leaf extract of *Heliotropium indicum* Linn. was found to be the most active, forming the largest inhibition zone (30 m.m.) in our experimentation., leaf extract of *Tectona grandis* Linn.,

Crinum latifolium linn, *Lowsonia inermis* linn.,and stem bark extract of *Mussaenda globosa, Acacia nilotica* Linn. and whole plant extracts of *Cynodan dactylon* L. *were* next to it forming the inhibition zone *(24 m.m.),* leaf extract of *Coccinia indica* and *Mimusops elengi* L. and root of *Boerhaavia diffusa* L. , *Solanum nigrum* L.formed a inhibition zone (18 m.m.). Other samples showed less or no inhibition zone in the experiments. The result indicate that the leaves of *Heliotropium indicum* Linn. can be exploited as a good source of innocuous bactericide. . Further studies on this plant are in progress.

Key words : Higher plant extract, *Antibacterial activity, Pseudomonas aeruginosa.*

Our earth, as an ecosystem, has so many abiotic and biotic components which are continuously in a state of action – reaction and interaction.Our environment has a fascinating world of microorganisms which not only affect our daily life but also natural resources. Disease control has undergone cyclic changes from very early times. The use of plants to control disease has been in practice since the days of the Ramayana era; Ayurveda and Charak Samhita are mainly based on herbal therapy. Excessive use of synthetic chemicals sometimes irreversibly damages the ecosystem, contaminates soil surface and ground water, and finally disturbs the food chain. Such chemicals have been found to exhibit teratogenicity, carcinogenicity, phytotoxicity, and residual effects[9,12]. In comparison to synthetic compounds the natural compounds provide less toxic. more systemic and easily biodegradable,free from environmental toxicity and have little or no side effect in human beings.[5,7,9]. This facts inspired us to investigte antibacterial activity in higher plants of this locality.

Collection and extraction: Fresh plant parts of 25 medicinal plants were collected randomly from district Azamgarh and Mau, India. The collected plant parts were thoroughly washed with sterilized water, and excess water was soaked off with the help of cheese cloth. 5.0g (fresh weight) of each part was macerated to pulp in a warring blender. The pulp was kept immersed in 25 ml of 50% ethanol (v/v) overnight at room temperature and finally filtered through filter paper. Maximum constituents of the plant parts dissolved in the solvent due to the maximum polarity of ethanol and water. The test pathogen *Ps*eudomonas aeruginosa was collected from the sputm of patient, cultured on **Mac Conkey's Agar Medium** and identified by different colony characters and biochemical test.

Disc preparation : 2.0 ml of the filtered extract was soaked into a disc (diameter 10 mm) of Whatman paper No. 1 with the help of a hair drier. The disk was dipped in a 2.0ml extract and dried under a hair dryer. The process was repeated with sterilized forceps until the whole extract was completely impregnated in the disk. These were designated as "**treatment disks**." Side by side, "**control disks**" were also prepared by impregnating 2.0ml of 50% (v/v) ethanol only onto the Whatman paper No. 1 disks.

Culture medium : Mac Conkey's agar medium was prepared and sterilized. The sterilized medium was poured into pre-sterilized petri plates (3 mm diameter) to cover the base of the plate.

Assay procedure : The treatment and control disks were assayed for antibacterial activity against the test pathogen by the "**Inhibition zone technique**". First of all Peotone water was prepared by dissolving **Peptone** (**1**.0 gram) and sodium chloride (0.5 gram) in **100 ml** water. The sterilized peptone water aseptically inoculated with the test pathogen, and incubated at 37°C for half an hour so that the inoculum may diffuse homogeneously in the peptone water. Thus prepared the 'seed' of test pathogen *Pseudomonas aeruginosa*. Now already prepared agar plate were seeded with the test pathogen by pouring aseptically 10 ml. of "seed" on each plate with the help of a sterilized pipette. These plates were left undisturbed for **10 minutes** during which the bacteria in the

solution settled on the surface of the solid medium. Then the supernated solution was decanted in detergent solution. The impregnated treatment disks were aseptically placed on the medium in the center of seeded culture plates. One treatment disk was placed on each plate. Control plates were prepared by placing control disks on the Mac Conkey's agar medium in the center.

Inoculation and Incubation : Both treatment and control plates were inoculated with the test pathogen *Pseudomonas aeruginosa*. Both treatment and control plates, were incubated at 37°C for 24 hours. For this purpose. Unless mentioned otherwise, the experiments were kept in three replicates and repeated twice to confirm the result.

Results were obtained by comparing the activity in treatment and control disks. The compounds in the impregnated treatment disks gradually diffused into the medium.. where the compound present in disk had antibacterial activity they interacted with the pathogen of seeded agar plate and checked the growth of the pathogen in the form of an inhibition zone around the disk. On the other hand no zone was found around the inactive treatment disk and similarly no inhibition zone formed around the control disk (figure 1). Inhibition zone of different diameter were formed in different treatment plates according to the degree of ANTIBACTERIAL ACTIVITY in impregnated disks. The inhibition zone on each plate was measured (diameter) in perpendicular direction and mean value was recorded. (Table-1).

The screening revealed that the leaf extract of *Heliotropium indicum* linn. Belonging to family Boraginaceae was found to be the most active, forming the largest inhibition zone (30 m.m.) in our experimentation., leaf extract of *Tectona grandis* linn., *Crinum latifolium* linn, *Lawsonia inermis* linn., and stem bark extract of *Mussaenda glabra* Hook*., Acacia nilotica linn.* and whole plant extracts of *Cynodan dactylon* L. *were* next to it forming the inhibition zone (24 m.m.), leaf extract of *Coccinia indica* and *Mimusops elengi* L. and root of *Boerhaavia diffusa* L., *Solanum nigrum* L. formed a (18 m.m.). Other samples showed less or no inhibition zone in the experiments. The result indicate that the leaves of *Heliotropium indicum* Linn. can be exploited as a good source of innocuous bactericide against the test pathogen *Pseudomonas aeruginosa.*

Table-1. Antibacterial activity of different plant extracts screened against test pathogen *Pseudomonas aeruginosa*

S. No.	Plant and Family	Plant part Used	Inhibition zone (m.m.)
1	*Acacia nilotica* L.*(Mimosaceae)*	Leaf	24.00
2	*Boerhaavia diffusa* L. *(Nyctaginaceae)*	Root	18.00
3	*Crinum latifolium.* L. (Amaryllidaceae)	Leaf	24.00
4	*Cynodon dactylon* L. (Graminae)	Leaf	2.00
5	*Argemone mexicana* L. (Papaveraceae)	Leaf	12.00
6	*Emblica officinalis* Gaertn (Euphorbiaceae)	Leaf	15.00

7	*Euphorbia hirta* L. (Euphorbiaceae)	Leaf	00.00
8	*Heliotropium indicum* L. (Boraginaceae)	Leaf	30.00
9	*Ipomoea fistulosa* Mart (Convolvulaceae)	Leaf	11.00
10	*Jatropha gossypifolia* L. (Euphorbiaceae)	Leaf	14.00
11	*Lawsonia inermis* L. (Lythraceae)	Leaf	24.00
12	*Madhuca indica* J.F. Gmel (Sapotaceae)	Leaf Stem bark	1300 14.00
13	*Mussaenda glabra* L. (Rubiaceae)	Leaf Stem bark	17.10 24.00
14	*Mentha piperita* L. (Labiatae)	Leaf	14.00
15	*Nicotiana plumbaginifolia* Viv (Solanaceae)	Leaf	15.00
16	*Pongamia pinnata* Forst.(Leguminosae)	Leaf Stem bark	14.00 16.00
17	*Punica granatum* L. (Punicaceae)	Leaf	17.10
18	*Rumex dentatus* L. (Polygonaceae)	Leaf	14.00
19	*Saraca indica* L. (Caesalpiniaceae)	Leaf Stem bark	16.00 00.00
20	*Solanum nigrum* L. (Solanaceae)	Leaf	18.00
21	*Tamarindus indica* L. (Caesalpiniaceae)	Leaf Stem bark	16.00 14.00
22	*Tectona* grandis L. (Verbenaceae)	Leaf Stem bark	24.00 16.00
23	*Typha australis* Schum (Typhaceae)	Leaf	00.00
24	*Thevetia peruviana* Pers. (Apocynaceae)	Leaf	15.00
25	*Xanthium strumarium* Ld (Compositae)	Leaf Root	14.00 15.00

Fraction of the ethanolic extracts :

It is a general principle that different organic solvents have different polarities, *i.e.* efficiency to dissolve specific compounds into them. On the basis of this principle the ethanolic extracts of active plants were treated with different organic solvents one by one. The petroleum ether fraction was found antibacterial activity against the test pathogen. Minimum Inhibitory concentration of Petroleum ether fraction was worked out, and it was found $1.2\mu g / m L$. At this MIC, the fraction was found to be antibacterial.

Comparison of Active fraction and Commercial Antibiotics for measuring the efficacy of Antibacterial activity :

The antibacterial activity of the active fraction,at MID, was compared with the activity of certain commercial antibiotics which are used to control different infections of Gram – positive and Gram – negative bacteria. Some

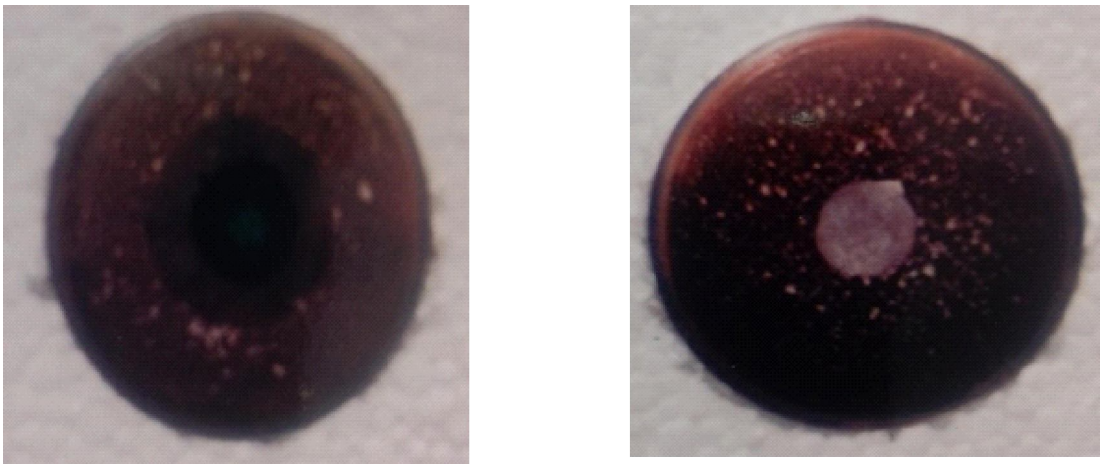

Fig. 1. (Treatment plate of active fraction showing large inhibition zone and control plate showing absence of inhibition zone.)

'microbial sensitive discs '(Span combi discs, code No. 14347, Spon diagnostics Ltd., Sachin -334 230, India) were selected for the comparison. The comparison was done by 'Antimicrobial susceptibility testing methods.

(Performance standards for antimicrobial discs susceptibility test – Fourth Edition, National committee for clinical laboratory standards vol. 13, No. 24, 1993). The test was based on the fact that for a given antibiotic, the size of the zone of inhibition is inversely related to the number of strains being tested when the test conditions are held constant. The active fraction disk (5.0 mm diameter) containing 9.0 mg. of the constituent of the active uhfraction was placed in the centre and the 7 sensitivity disks were placed equidistantly in the surface of 'seeded' Mac Conkey's agar medium plate with the help of a flamed forceps. Allowed the plate to stand at room temperature for 30 minutes (prediffusion time) and after that it was incubated at 37°C. The zone of inhibition, if any, were measured and recorded after 24 hours. The observation recorded. The result indicate that the inhibition zone around the active fraction disk was largest. Ciprofloxacin, Cefotaxime and Cephalexin inhibited the test pathogen upto some extent. However, Ampicillin, Cephadroxil, Co-trimoxazol and Lincomycin were almost inactive and did not inhibite the test pathogen upto significant level. Over all the active fraction of ***Heliotropium indicum*** Linn. at its MID was found to be more active than commercial antibiotics tested. (Fig. 2).

Thus, the leaf extract of *Heliotropium indicum* Linn can be exploited as a good source of herbal antibiotics against the test pathogen *Pseudomonas aeruginosa* Various workers have screened a large number of plants belonging to angiosperms for their antimicrobial properties. Mostly, aqueous extracts or expressed juices of plants have been used to evaluate antifungal and antibacterial activity[8,11]. However, some workers have used organic extractives in addition to crude and

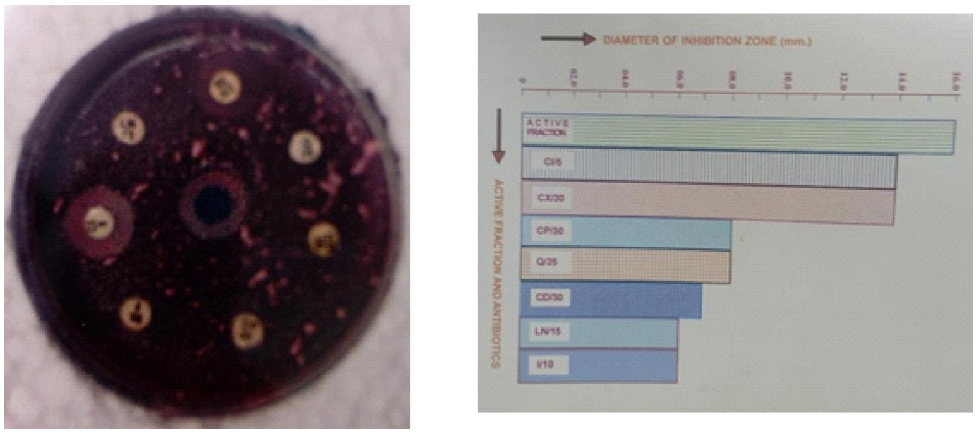

Fig. 2. Showing comparision of commercial antibiotics with active fraction.

aqueous extract.[1,8,11]. During the course of the present assay, 50% ethanol was used as an extractive. The use of 50% ethanol ensures extraction of maximum compounds due to its high polari the sty[3,9]. Aqueous extracts of expressed juices may lose their efficacy due to degradation of active constituents by continued enzymatic activity.

We are thankful to Dr. Tausif. Ahmad, Professor and Head of Department of Botany, S.N.P.G. College Azamgarh, and Director Dr. Shudhanshu Shekhar, R.S. College of Pharmacy for providing laboratory facilities and thankful to Dr Heera Lal Yadav and Dr. Harendra Yadav. M.D. Nobel Hospital Azamgarh for their valuable suggestions.

References :

1. Ali, M.I.A., N.M.M. Shalaby, M.H.A. Elgamal and A.S.M. Morige (1999). *Phytotherapy Research*, *13:* 401-407.
2. Dixit, S.N., S.C. Tripathi and R.R. Upadhay (1976). The antifungal substance of rose flower (*Rosa indica*), *Economic Botany*, *30:* 371-374.
3. Dhar, M.L., B.N. Dhawan, B.N. Mehrotra and C. Ray (1973). *Indian J. Exp. Biol., 6:* 232-247.
4. Fawcett, C.H. and D.M. Spencer, (1970). *Ann. Rev. Phytopathol 8:* 403-418.
5. Heeralal, *et al*. (2010). *Indian J. Sci. Res. 2:* 111-115.
6. Hooda, K.S. and M.P. Srivastava (1998). *Indian J. Plant Pathology*, *16:* 64-66.
7. Kumar, A., S.K. Roy, D.C. Saxena, and A.R. Saxena (1995): *Neo Botanica*, *3:* 1-2.
8. Khan, M.R., M. Kihara, and A.D. Omyon (2001). *Fitoterapia*, *72:* 174-176.
9. Saxena A.R., R.K. Yadav, H.L. Yadav, R. Sahani, Madhri Saxena and K. Kirti (2002). *Neo Botanica 10:* 21-30.
10. Tripathi S.C. and S.N. Dixit (1977). *Experientia 33:* 207.
11. Srivastava, Deepak and Yadav Heeralal (2008). *Indian Phytopath*, *61*(1): 99-102.
12. Yadav, H.L., A.R. Saxena and SJ. Ali (2007). *Indian J. Appl. and Pure Bio. 22*(2): 315-316.