Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6094

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (1):** 461-466 (2018)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Antimicrobial Activity of Some Indian Medicinal Plants against the Soil Bacteria

Mukesh R. Jangra[1], Sumit Jangra[2*], Raj Kumar[3] and K. S. Nehra[1]

[1]Department of Biotechnology, Government College, Hisar- 125 001

[2]Department of Molecular Biology, Biotechnology and Bioinformatics

CCS Haryana Agricultural University, Hisar- 125 004

[3]Department of Botany, Government College, Hisar- 125 001

*Corresponding Author E-mail: sumit.jangra712@gmail.com

Received: 13.12.2017 | Revised: 16.01.2018 | Accepted: 24.01.2018

ABSTRACT

In the present study, soil samples were collected from hospital nearby areas and some bacteria were isolated and purified by using spread plate and streak plate methods. The six strains named A, B, C, D, E and F were cultured. Cultural, morphological and biochemical characterization was done to identify theses bacterial isolates. Eighteen Indian medicinal plantswere selected to examine the antimicrobial activity against the cultured species using disk diffusion method. Methanolic extraction as well as water extract of the plant leaves and roots was prepared and phytochemical screening of these plants was performed for constituents such as flavonoids, saponins, glycosides, tannins, phytosteroid, phenols, alkaloids, etc. Among all the plants Citrus Limon showed maximum antimicrobial activity (by inhibition zone method) against the isolated microbes. However, the isolated microbes were susceptible to the methanolic extracts of the above mentioned plants. In future, these plants can be further subjected to isolation of the therapeutic antimicrobials for health purposes and food applications.

Key words: *Microorganism, Antimicrobial activity, Saponins, Glycosides, Tannins, Phytosteroid*

INTRODUCTION

A huge diversity of microorganism including bacteria are present in the soil. Our environment is surrounded with a large number of microorganism among which bacteria are the most important and abundant. These are small in size, unicellular, prehistoric and non-photosynthetic microorganism. Among the various methods employed for isolating bacteria from soil, dilution is the most important. Soil microbiota represents one of the primitive evolutionary origins of antibiotic resistance and is a reservoir of resistance genes available for exchange with clinical pathogens[3], A gram of soil may contain up to 5000 or more different species of bacteria[7], Multidrug resistant strains of pathogenic bacteria such as *Escherichia coli* and *Klebsiella pneuminiae* are prominent in hospital areas and are increasing being isolated from community acquired infections[13], *Pseudomonas aeruginosa* can be found in nature from sources as diverse as water, soil and plants.

Cite this article: Jangra, M.R., Jangra, S., Kumar, R. and Nehra, K.S., Antimicrobial activity of some Indian medicinal plants against the soil bacteria, *Int. J. Pure App. Biosci.* **6(1):** 461-466 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6094

P. aeruginosa is more widely known as an opportunistic pathogen for humans and animals than as soil bacteria and can produce severe infections in immune-compromised hosts[6]. So, soil from the hospital nearby areas was thought to be the suitable source for the isolation of pathogenic bacteria. Biochemical test is used to differentiate among the other bacteria. Since long, natural products from plants have played a key role in maintaining human health and in the past few years there has been a great emphasis on natural therapies. In many countries the use of pharmaceutical based on photochemicals has increased gradually nowadays. Reports from WHO says that medicinal plants would be the finest source to obtain a diverse range of drugs. In developed countries nearly 80% individuals use traditional medicines, which possess products obtained from medicinal plants. The therapeutic treatment based on the crude extract obtained from medicinal plants and phytochemicals of known antimicrobial activity is of great significance. The efficacy of therapeutic treatments based on medicinal plants is proved by various studied conducted in different countries in recent years. Plants are rich in wide variety of secondary metabolites known as phytochemicals such as tannins, flavonoids, steroids, terpenoids, napthoquinone, inulin, alkaloids, water soluble phenols and phlobtannins. These phytochemicals have wide applications from the ancient times in the treatment of infectious diseases and have fewer side effects and reduced toxicity. Previous studies were carried out on the antimicrobial activity of methanolic extracts of *Acacianilotica*, *Sidacordifolia*, *Tinosporacordifolia*, *Withania somnifer* and *Ziziphus mauritiana* and they showed significant antimicrobial activity[5], A study reported that *Cinnamomum zeylanicum* bark can be used to design reliable and safer herbal drug and could be used in the pharmaceutical preparations for the treatment of infectious and malignant diseases[12], Afolayan (2003) tested the antimicrobial activity of plants and showed that plants are a potential source of innovative antibiotic prototype. In the recent years incidence of multiple resistance in human pathogenic microorganisms has increased, mainly because of random use of commercial antibiotics, generally used in treatment of infectious diseases. This has forced scientific community to think about novel antimicrobial substances from different sources like medicinal plants[9,10], A wide range of secondary metabolites are produced by plants which can be directly used as precursors or as principal compound for drug synthesis in pharmaceutical industry[8], It is anticipated that plant extracts exhibiting target sites other than those used by antibiotics be effective against microbial pathogens which are drug resistant. However, a very little is known about such activity of medicinal plants and from a huge diversity of plant species (nearly 4, 00,000) only a few has been scientifically examined for their antimicrobial activity[1], Bioactive compounds are normally accumulated as secondary metabolites in all plant cell but the concentration is determined by the plant part, climate and growth phase. The highest concentration of such compounds is present in leafs generally preferred for therapeutic use. The growth of disease causing microbes is inhibited by these bioactive compounds, either singly or in combination. The proposed project would, therefore, be directed to check the antibacterial activity of some Indian medicinal herbs against the bacterial strains isolated from soil and with following objectives: 1) Isolation and characterization of soil bacteria; 2) To determine the susceptibility of test bacteria to the crude extracts of medicinal plants; 3) To determine the zone of inhibition of plant extract on test bacteria; 4) Photochemical analysis of the plant extracts.

MATERIAL AND METHODS

Sampling site and sample collection

The micro organisms are derived from their natural habitat. In the present study, soil samples were collected from hospital nearby areas from Hisar, Haryana, India. The leaves, fruit pulps and seeds of selected plants were collected from Medicinal, Aromatic and Potential Crops Section, Department of

Genetics and Plant Breeding, College of Agriculture, CCS HAU, Hisar.

Isolation of bacteria

For bacterial isolation, 10g of soil was collected from public garbage dumping area. Sterile and clean spatula was used to collect the soil sample in fresh polythene, brought to the laboratory and stored overnight at 4°C. For reducing the microbial load, soil suspension was made by dissolving 1 g of soil in 10 ml of sterile distill water. A dilution of 10^{-6} was obtained by serial dilution. 1 ml each from 10^{-4},10^{-5} and10^{-6} dilutions were spreaded on the agar plates using sterilized spreader. The incubation time and temperature for culture growth was kept 48 hrs and 37 ˚C respectively to have well isolated colonies. Single colony was isolated using serial dilution method. The bacterial cells were cultured on LB agar nutrient media. On the basis of shape structure and color of bacterial colony, 6 bacterial pure culture was isolated by series of streaking, named A, B, C, D, E and F were selected for further experimentation. The plant samples were washed dried in hot air oven maintained at 40- 50 ˚C for 24-48 hrs. 100 g of the dried sample was extracted with methanol. The dried plant extract was then dissolved in DMSO (Dimethyl sulfoxide) and stored at -80 ˚C for further use.

Biochemical and Confirmatory tests of isolates

Gram staining technique was applied to differentiate the isolated bacterial species. Biochemical tests such as Catalase test, Indole test, Methyl Red test, Voges-Proskauer test and Citrate test were carried out for the identification of the isolates[4], Confirmatory test by observing the growth of the isolates on Mannitol salt agar and Nutrient broth were also performed to aid the identification process.

Antibiotic sensitivity of the isolates

The isolated bacterial cultures were inoculated in Nutrient broth and kept overnight in incubator at room temperature for 3-5 days. Nutrient agar was prepared and autoclaved for 15-20 min at 15 psi. The agar was poured in sterile petriplates and kept for cooling. Antibiotic sensitivity test was done to check the sensitivity of the isolates against 3 antibiotics namely Ampicillin, Streptomycin and Kanamycin.

Phytochemical screening of plant extracts

The dried plant extract was tested for the presence of phytochemicals. Phytochemicals screening tests were done for the presence of flavonoids, saponins, tannins, steroids, terpenoids, alkaloids, water soluble and phenols[2], Additional tests such as Iodine test, Ninhydrin test; Molish test and Benedict's test were also checked for the presence of proteins, starch, amino acids, carbohydrates and reducing sugar respectively.

Assay of antimicrobial activity using Disc diffusion method

Herbal mixtures made for plant parts are contributing a lot to human health and well-being, so they have provided a source of hope for development of novel drug compounds. The role of plant extracts with known antimicrobial properties in therapeutic treatment is of great significance. Agar plates were inoculated with standardized inoculums of the test microorganism. Then the filter paper disc (2 cm in diameter) was placed on the agar surface containing the test bacterial isolates at desired concentration[11], The petri plates were incubated under appropriate conditions. Usually antimicrobial agents inhibits the germination and growth of test microorganism by diffusing into the agar and the inhibition diameter zone was measured thereafter. The plates were examined for the presence of bacterial growth inhibition which was depicted by the clear zone around the disc. The inhibition zone size (including disc) was assessed in millimeters. The absence of activity was interpreted by the zone of inhibition. All experiments were carried out under strict ascetic conditions and were replicated thrice. If the zone of inhibition was less than 7 mm than the activity is expressed as resistant, intermediate when zone was about 8 mm and sensitive if more than 11 mm.

RESULTS AND DISCUSSION

Isolation and identification of isolates

On the basis of morphological and cultural tests, it was observed that the most bacterial colonies appear white or a creamy yellow in color, and are fairly circular in shape. Bacterial strain A was creamish white in colour with smooth surface, circular form, raised elevation, smooth borders and of small size. B strain was deep yellow in colour with smooth surface, circular form, raised elevation, smooth borders and of small size. C bacterial strain was light yellow in colour with smooth surface, circular form, raised elevation, smooth borders and of medium size. D bacterial strain was cream in colour with smooth surface, circular form, flat elevation, smooth borders and of small size. E bacterial strain was cream in colour with rough surface, circular form, flat elevation, smooth borders and of small size. F bacterial strain was orange in colour with smooth surface, circular form, raised elevation, smooth borders and of large size (table 1).

Table 1: Morphological and cultural tests

Sr. No.	Character/Bacteria strains → ↓	A	B	C	D	E	F
1	Colour	Creamish white	Deep yellow	Light yellow	Cream	Cream	Orange
2	Surface	Smooth	Smooth	Smooth	Smooth	Rough	Smooth
3	Form	Circular	Circular	Circular	Circular	Circular	Circular
4	Size	Small	Small	Medium	Small	Small	Large
5	Elevation	Raised	Raised	Raised	Flat	Flat	Raised
6	Edge	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
7	Gram stain	Positive	Negative	Positive	Negative	Negative	Negative

Table 2: The result of biochemical test

Sr.no	Bacterial culture/ Tests → ↓	A	B	C	D	E	F
1.	Indole Test	(-)	(-)	(-)	(-)	(-)	(-)
2.	Methyl Red Test	(-)	(-)	(-)	(-)	(+)	(+)
3	Voges-Proskauer Test	(+)	(-)	(+)	(+)	(+)	(+)
4	Citrate Utilisation Test	(-)	(-)	(+)	(-)	(+)	(+)
5	Mannitol Salt Agar Test	(+)	(-)	(-)	(+)	(-)	(-)
6	Catalase Production Test	(+)	(-)	(+)	(+)	(-)	(+)
7	Antibiotic Resistance Test	(-)	(-)	(-)	(-)	(-)	(-)
8	Gelatin Hydrolysis Test	(+)	(+)	(+)	(+)	(-)	(+)
9	Urease Test	(-)	(-)	(-)	(-)	(-)	(+)
10	Cellulose Hydrolysis	(-)	(-)	(-)	(-)	(-)	(-)
11	Carohydrate Fermentation Test • Glucose • Lactose • Sucrose • Fructose	 (+) (-) (-) (-)	 (+) (-) (-) (-)	 (+) (-) (-) (-)	 (+) (-) (-) (-)	 (+) (-) (-) (-)	 (+) (-) (-) (-)

Antibiotic resistance test

All strains were sensitive to antibiotics, hence no one the isolates were able to grow in the presence of Ampicillin, Streptomycin and Kanamycin. Cycloheximide was used to check whether the strains are of bacteria or not as it is active against fungi only. All bacterial strains were resistance to cycloheximide

Antibacterial activity of extracts

The antimicrobial activity of Methanolic extracts of *Phyllanthus emblica* on different pathogenic organisms using disc diffusion method have showed maximum zone of inhibition (table 3) against *strain (15mm)* followed by strain *D* (11mm), *strain A* (10mm) and *strain F* (10mm). Similarly, the pure extract of *Citrus limon* fruit juice show maximum zone of inhibition against strain A and D (15mm each), followed by strain *D* (14mm), *strain* B (10mm) and *strain C*(9mm). The collective analysis of antimicrobial activity of methanolic extract indicated that among the seven medicinal plants used in the study *Phyllanthus emblica* and *Citrus Limon* have better impact ranged from 9 to 15mm on all the species of bacterial strains when compared to rest of the plant species such as *A. indica*, *S. aromaticum*, *A. leucophora* and

C. longa (ranged from 3 to 8mm). Whereas, other plant extracts also showed irregular/least even no zone of inhibition against selected soil bacteria in case of agar disc diffusion method (table 3). The antibacterial activity of above all plant extracts were also checked against mix culture there were no positive results noticed.

Table 3: Zone of inhibition shown by different plants

Strains → / Plants ↓	A	B	C	D	E	F
Azadirachtaindica	4mm	nil	nil	5mm	2mm	3mm
Curcuma longa	2mm	1mm	6mm	2mm	1mm	3mm
Allium sativum	nil	4mm	2mm	nil	2mm	nil
Zingiberofficinale	1mm	2mm	4mm	2mm	2mm	1mm
Leucaszeylanica	nil	nil	nil	nil	nil	1mm
Phyllanthusemblica(fruit)	6mm	nil	7mm	11mm	9mm	5mm
Phyllanthusemblica (powder)	10mm	15mm	9mm	14mm	11mm	10mm
Calotropis procera	nil	nil	2mm	Nil	5mm	3mm
Acacia leucophloea	irregular	2mm	3mm	5mm	3mm	6mm
Cuscutareflexa	2mm	2mm	nil	nil	nil	nil
Calendula officinalis	nil	1mm	nil	Nil	1mm	nil
Menthaarvensis	nil	nil	1mm	Nil	1mm	nil
Cinnamomumzeylanicum	4mm	4mm	nil	2mm	nil	nil
Lantana camara	nil	nil	1mm	irregular	1mm	nil
Syzygiumaromaticum	4mm	7mm	2mm	5mm	5mm	4mm
Citrus limon	15mm	10mm	8mm	15mm	12mm	14mm
Brassica campestris	1mm	nil	nil	nil	1mm	nil
Tinosporacordifolia	irregular	2mm	irregular	irregular	irregular	irregular
Cannabis sativa	nil	1mm	1mm	nil	nil	nil

Phytochemical test on Herbs

Medicinal herbs contain a number of secondary metabolites like alkaloids, tannins, flavonoids, saponin, phenols, phytosterols, proteins, quinones etc. which serves as antimicrobial activity in them. For the detection of presence of secondary metabolites certain phytochemical tests were carried out, results are given in table 4.

Table 4: Phytochemical analysis of different plants

S.No.	Samples	Phytochemical test							
		Alkaloids	**Tanninn**	**Saponnin**	**Flavanoids**	**Phenols**	**Phytosterol**	**Proteins**	**Carbohydrates**
1	Azadirachtaindica	-	+	+	-	+	+	-	+
2	Cinnamomumzeylanicum	-	-	+	-	-	-	-	+
3	Chlorophytumborivilianum	-	+	-	-	+	-	-	+
4	Syzygiumaromaticum	+	+	+	+	+	-	-	+
5	Curcuma longa	+	+	-	-	+	-	-	+
6	Allium sativum	-	-	+	-	-	-	-	+
7	Zingiberofficinale	-	-	+	+	-	-	-	+
8	Phyllanthusemblica	-	-	-	+	-	-	+	-
9	Calotropisprocera	-	+	-	-	+	-	-	+
10	Leucaszeylanica	+	-	-	+	-	-	-	+
11	Acacia leucophloea	-	+	+	-	+	-	-	+
12	Brassica campasteris	+	-	+	-	-	+	-	-
13	Cuscutareflexa	-	+	+	+	+	-	+	-
14	Calendula officinalis	-	+	-	+	+	-	-	+
15	Menthaarvensis	-	-	+	-	-	-	-	+
16	Cannabis sativa	+	+	+	-	-	-	-	+
17	Lantana camara	-	+	+	+	-	-	-	+
18	Citrus limon	-	-	+	-	-	-	-	+
19	Tinosporacordifolia	-	-	+	-	-	-	+	+

CONCLUSIONS

This study concludes that many Indian herbs can be used against the multidrug resistant or pathogenic bacteria. So, there is a clear need for exploration of new antimicrobial agents with novel mode of action from plant sources and to study the potentiality for applications in food systems. Considering the impact of the antibiotics on pathogens and normal flora, search for potential antimicrobial agents from plant sources is a good alternative aspect. Pharmacological evaluations and understanding the mechanism of these biologically active compounds in the inhibition of the pathogens is a promising area of research. Future research is also necessary to use the phytochemicals as food additives or as functional ingredients in food.

REFERENCES

1. Balandrin, M.F., Klocke, J.A., Wurtele, E.S. and Bollinger, W.H. Natural plant chemicals: Sources of Industrial and Medicinal materials. *Science*. **228:** 1154-1160 (1985).
2. Evans, W.C. Trease and Evans' pharmacognosy. 16th edn. London: Elsevier Health Sciences. (2009).
3. Forsberg, K.J., Reyes, A., Wang, B., Selleck, E.M., Sommer, M.O. and Dantas, G. The shared antibiotic resistome of soil bacteria and human pathogens. *Science*. **337(6098):**1107-1111 (2012).
4. Holding, A.J. and Collee, J.G. Routine biochemical tests. London and New York: Academic Press (1971).
5. Mahesh, B. and Satish, S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. *WJAS*. **4(5):** 839-843 (2008).
6. Quinn, J.P. Clinical problems posed by multiresistant nonfermenting gram-negative pathogens. *Clinical Infectious Diseases*. **27(1):** 117-124 (1998).
7. Schloss, P.D. and Handelsman, J. Metagenomics for studying unculturable microorganisms: cutting the Gordian knot. *Genome Biology*. **6(8):** 229-229 (2005).
8. Srivastava, J., Lambert, J. and Vietmeyer N. Medicinal plants: An expanding role in development. World Bank Technical Paper. No. 320 (1996).
9. Stockwell, C. Nature's pharmacy. London, United Kingdom. Century Hutchinson Ltd.
10. Thomson, W.A.R. 1978. Medicines from the Earth. Maidenhead, United Kingdom. McGraw-Hill Book Co (1988).
11. Vanden Berghe, D.A. and Vlietinck, A.J. Screening methods for antibacterial and antiviral agents from higher plants. In: Dey, P.M., Harbone, J.D. (eds), Methods in Plant Biochemistry, Academic Press, London, *p.* 47–69 (1991).
12. Varalakshmi, B., Anand, A.V., Karpagam, T., Bai, J.S. and Manikandan, R. In vitro antimicrobial and anticancer activity of *Cinnamomum zeylanicum* Linn bark extracts. *Int J Pharm Pharm Sci*. **6(1):** 12-18 (2014).
13. Wiener, J., Quinn, J.P., Bradford, P.A., Goering, R.V., Nathan, C. and Bush, K. Multiple antibiotic–resistant Klebsiella and *Escherichia coli* in nursing homes. *Jama*. **281(6):** 517-523 (1999).