

Original Research Article

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Evaluation of Antibacterial Activity of Plant Extracts on Antibiotic Resistant Bacteria

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ABSTRACT

The antimicrobial activity of plant extracts (crude extract of leaves) were evaluated against antibiotic-resistant bacteria. Crude extracts of *Acacia arabica*, *Aegle marmelos*, *Ailanthus excelsa*, *Alstonia scholaris*, *Callistemon lanceolantus*, *Casurina equisetifolia*, *Catharanthus roseus*, *Grevillia robusta*, *Kalanchoe pinnata*, *Moringa oleifera*, *Psidium guajava*, *Saraca indica*, *Tamarindus indicum*, *Thuja compacta* and *Vitex negundo* were selected. The plant extracts were tested against the following microorganism: *Escherichia coli* NCIM 2931, *Proteus vulgaris* NCIM 2857, *Pseudomonas aeruginosa* NCIM 5029, *Bacillus cereus* NCIM 2155, *Bacillus subtilis* NCIM 2063, *Bacillus megaterium* NCIM 2087, *Klebsiella pneumoniae* MTCC 432, *Proteus mirabilis* MTCC 425, *Salmonella typhimurium* MTCC 98, *Salmonella typhi* MTCC 734, *Staphylococcus aureus* MTCC 96, and *Staphylococcus epidermidis* MTCC 43. Bacteria which were resistant to some common antibiotics (Cephalothin, Cloxacillin, Erythromycin, Gentamicin, Methicillin, Gentifloxacin, Levofloxacin, Oleandomycin, Vancomycin), were found to be susceptible to crude extract of plants studied. *Ailanthus excelsa*, *Casurina equisetifolia*, *Kalanchoe pinnata*, *Moringa oleifera* and *Tamarindus indicum* showed significant antimicrobial activity against bacteria studied.

Keywords

Antimicrobial activity, Plant extracts, Antibiotic resistant bacteria

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Introduction

It is now common that market is receiving new antibiotics everyday to tackle the problem of drug resistance developed by pathogenic microorganisms. All pharmaceutical companies are reserving substantial part of their annual budget for developing new medicines. Clinical history of the disease is also changing due to development of new bacterial strains. Thus

patients have become victim of these new drugs which are mostly tried on trial and error basis. The patients have to bear the side effects of such antibiotics such as suppressed immunity, hypersensitive reactions, stomach upsets, etc.

Plants have been used for centuries to treat infectious diseases and are considered as an important source of new antimicrobial agents (Bereksi *et al.*, 2018). Several works have

been done to examine the antimicrobial effects of herbal plants extracts, including roots, stem, leaves or flowers (Cowan 1999; Kakad *et al.*, 2015)

Plant used for traditional medicine contains wide range of substances that can be used to treat chronic and infectious diseases (Alam *et al.*, 2009).

Various plants (referred as herbal) are important in human health care and several herbal products are being used by people for various health benefits. The world health organization (WHO) estimated that about 80% of the population living in developing countries relies almost exclusively on traditional medicine for their primary health care needs (Mukherjee 2005) therefore more work is required to investigate various properties, safety and efficiency of medicinal plants (Ellof, 1998.).

Several scientists have worked on antimicrobial activities of various medicinal plants and found that these plants are effective against the microorganisms which showed drug resistance to various antibiotics (Gislene 2000). Numbers of medicinal plants were screened for their antimicrobial activity. Many workers have studied the effect of different plant extracts on bacteria producing diseases in human being (Dulger and Gonuz, 2004).

Present study involves the screening of medicinal plants having potential antimicrobial activity against those bacteria which showed antibiotic resistance against common antibiotics.

Our study deals with the crude extracts of leaves of different medicinal plants. It is always successful to test crude extract as a tool in primary screening program than to screened pure components isolated from

natural products (Kusumto and Nakabayashi, 1995).

Materials and Methods

Test plants used in experiment

The following lists the plant of interest and family: *Acacia arabica*, (Family – Mimosoideae), *Aegle marmelos* (Family- Rutaceae), *Ailanthus excelsa* (Family – Simarubiaceae), *Alstonia scholaris* (Family – Apocynaceae), *Callistemom lanceolantus* (Family- Myrtaceae), *Casurina equisitifolia* (Family – Casuarinaceae), *Catharanthus roseus* (Family – Apocynaceae), *Grevellia robusta* (Family – Proteaceae), *Kalanchoe Pinnata* (Family – Crassulaceae), *Moringa oleifera* (Family – Morinagaceae), *Psidium guajava* (Family – Myrtaceae), *Saraca indica* (Family – Leguminosae), *Tamarindus indicum* (Family – Leguminosae), *Thuja compacta* (Family – Cupressaceae) and *Vitex negundo* (Family – Verbanaceae).

Bacterial cultures used:

Escherichia coli NCIM 2931, *Bacillus cereus* NCIM 2155, *Bacillus subtilis* NCIM 2063, *Bacillus megaterium* NCIM 2087, *Proteus vulgaris* NCIM 2857, *Pseudomonas aeruginosa* NCIM 5029, *Klebsiella pneumoniae* MTCC 432, *Proteus mirabilis* MTCC 425, *Salmonella typhimurium* MTCC98, *Salmonella typhi* MTCC734, *Staphylococcus aureus* MTCC 96, *Staphylococcus epidermis* MTCC 435.

The following antibiotics were used in the present study, Cephalothin (Ch30), Cloxacillin (Cx1), Erythromycin (E5), Gentamicin (G10), Gentifloxacin (Gf 5), Levofloxacin (Lo10), Methicillin (M5), Netilmicin sulphate (Nt30), Oleandomycin (OI15), Vancomycin (Va5).

These antibiotics were procured from HI-media Mumbai, India.

Hi -Sensitivity test broth (M 486) and Hi-sensitivity test agar (M 485) were procured from Hi-media Mumbai. The media were prepared according to the instructions given.

Agar cup method

Preparation of inoculum

A loopful of culture was inoculated from the stock slant culture in 5 ml of Hi-sensitivity test broth and broth was incubated at $35\pm 0.50^{\circ}\text{C}$ in BOD incubator for 18-20 hrs.

After incubation a loopful of actively growing culture was inoculated into 10 ml of Hi-sensitivity broth. Broth was incubated at $35\pm 0.50^{\circ}\text{C}$ for 6-8 hours. This culture was used for the inoculation of Hi-sensitivity test agar plates.

Preparation of Hi-sensitivity test agar medium

Hi-sensitivity test agar medium was prepared as per instructions of manufacturer. Required amount of agar medium was melted and 25 ml of molten medium was distributed in test tubes (25x150 mm). Medium was autoclaved at 15 lb. for 20 min. After autoclaving, medium was maintained at $45-50^{\circ}\text{C}$ in constant temperature water bath.

Inoculation of medium with test organism

A 0.5 ml of 6-8 hours old test organism is transferred to petridish (Sterilized in oven at 180°C for 1 hr.) using sterile micropipette. Hi-sensitivity test agar medium maintained at $45-50^{\circ}\text{C}$ was poured and mixed properly to ensure uniform distribution of organism with medium. Seeded plates are allowed to set at room temperature.

Preparation of agar cup

10 mm borer was used to cut cup in agar.

Four cups per plate at four equidistant corners were made.

A 100 μl crude extract was transferred by micropipette per well. Plates were immediately kept at 4°C in refrigerator for 1 hr. and then shifted to $35\pm 0.5^{\circ}\text{C}$ in BOD incubator. Zone of inhibition was measured after 24 hrs. of incubation.

Preparation of crude extract

Different plant leaves were collected from around Nagpur region in the month of August-September. Leaves were cleaned under running potable water and cut into pieces and grounded in pestle and mortar (made up of dolerite stone) till homogenized mass was obtained. Homogenized mass was squeezed in 400 mesh nylon cloth (pore size 37 micron) to obtain crude extract. Crude extract was kept in sterilized glass bottle. All crude extract were prepared fresh and used before 2 hours. Cold extracts were prepared using individual fresh plant leaves.

Antibacterial susceptibility test

Preparation of inoculum medium and inoculation of test organism were repeated as discussed. Antibiotic discs, four per plate were placed at four equidistant corners. Plates were incubated at $35\pm 0.5^{\circ}\text{C}$ and zone of inhibition were read after 24 hrs.

Results and Discussion

Total 12 bacteria were used for the present study. Susceptibility of these bacteria were tested against common antibiotics. The bacteria showed resistance to variable degree to the antibiotics i.e. Cephalothrin (Ch^{30}), Cloxacillin (Cx^1), Erythromycin (E^5), Gentamicin (G^{10}), Gentifloxacin (Gf^5), Levofloxacin (Lo^{10}), Methicillin (M^5), Netilmicin sulfate (Nt^{30}), Oleandomycin

(Ol¹⁵), Vancomycin (Va⁵). The results are given in Table 1. Total 15 plants were selected for the study. The 100 microlitre of

crude extracts of leaves of each plant were tested against 12 bacteria. The zones of inhibition are given in Table 2.

Table.1 Susceptibility of test organisms against antibiotics (zone in mm)

Antibiotics	EC NCIM 2931	ST MT CC 734	STM MTCC 98	KP MTCC 432	PV NCIM 2857	PM MT CC 425	PA NCI M 5029	SA MT CC 96	SE MT CC 435	BC NCIM 2155	BS NCIM 2063	BM NCIM 2087
Cephalothin (Ch30)	10	17	19	14	--	17	--	25	30	10	50	46
Cloxacillin (Cx1)	--	--	--	--	--	--	--	22	24	30	29	33
Erythromycin (E5)	--	--	--	--	--	--	--	14	21	21	28	21
Gentamicin (G10)	12	16	14	14	13	12	27	18	23	19	37	21
Gentifloxacin (Gf 5)	22	23	19	15	26	18	37	21	24	18	48	50
Levofloxacin (Lo10)	24	20	21	19	22	19	29	22	25	25	40	39
Methicillin (M5)	--	--	--	--	--	--	--	18	20	25	30	27
Netilmicin sulphate (Nt30)	18	21	17	12	16	11	26	15	23	22	34	37
Oleadomycin (OI15)	--	--	--	--	--	--	--	14	20	21	21	21
Vancomycin (Va5)	--	--	--	--	--	--	--	12	15	17	22	16

Table.2 Antimicrobial activity of crude extract against test organisms

Plants	EC NCIM 2931	ST MTCC 734	STM MTCC 98	KP MTCC 432	PV NCIM 2857	PM MTCC 425	PA NCIM 5029	SA MTCC 96	SE MTCC 435	BC NCIM 2155	BS NCIM 2063	BM NCIM 2087
<i>Acacia arabica</i>	--	--	17	--	12	14	--	20	16	23	14	13
<i>Aegle marmelos</i>	34	35	25	28	32	32	13	--	--	--	--	--
<i>Ailanthus excelsa</i>	18	15	20	22	13	28	11	16	--	--	12	--
<i>Alstonia scholaris</i>	33	--	23	28	23	25	--	--	--	--	18	15
<i>Callistemon Lanceolantus</i>	--	--	--	13	16	--	20	17	--	22	16	16
<i>Casurina equisitifolia</i>	28	--	22	27	22	30	--	17	15	22	13	13
<i>Catharanthus roseus</i>	29	--	17	26	25	16	--	--	--	--	22	19
<i>Grevillia robusta</i>	35	--	16	20	--	27	15	--	--	13	27	20
<i>Kalanchoe Pinnata</i>	31	13	32	24	29	31	12	--	--	13	23	--
<i>Moringa Oleifera</i>	21	--	16	23	21	21	--	22	23	25	18	21
<i>Psidium guajava</i>	20	15	--	--	27	18	--	16	--	20	13	12
<i>Saraca indica</i>	--	--	--	18	--	12	--	20	14	25	28	23
<i>Tamarindus indicum</i>	14	19	12	12	18	14	15	15	17	23	23	22
<i>Thuja compacta</i>	--	--	20	20	11	15	13	--	--	13	16	11
<i>Vitex negundo</i>	26	--	16	23	--	--	--	--	13	19	16	18

Most studies show that crude extract of leaves of *Acacia arabica* are active against several types of cariogenic bacteria found in human oral cavity (Akpatá and Akinremisi, 1997). In present study, extract of leaves of *Acacia arabica* found to be inhibited against both Gram-positive as well as some Gram-negative organisms but *Escherichia coli*, *salmonella typhi*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were found resistant. *Aegle marmelos* was found very effective against all Gram negative organisms but all Gram positive organisms were found resistant. It shows good inhibitory effect against *Bacillus cereus*, *B.subtilis*, *S.aereus* in petroleum ether (Shukla and Mehta 2007). The methanol extracts of *Aegle marmelos* also shows great effect against both Gram positive and Gram negative organisms (Suresh *et al.*, 2009). *Staphylococcus* strains are of great concern as several of its strains are drug resistant (Lechner 2004). The crude extracts of leaves of *Ailanthus excelsa* showed inhibitory effect against all Gram negative organisms and *Staphylococcus aureus*. The chloroform extract of this plant shows a effective result against bacteria and fungi like *Aspergillus niger*, *A. fumigatus*, *Penicillium frequentence*, *P. notatum* and *Botrytis cinerea* (Joshi *et al.*, 2003). In the present study *Saraca indica* showed inhibition against *Klebsiella pneumoniae*, *Proteus mirabilis* and all Gram-positive organisms. The petroleum ether extracts of *Saraca indica* leaves show great effects against larvae of *Culex quinquefasciatus* with LC50 values 228.9 and 291.5ppm (Mathew *et al.*, 2008). *Callistemom lanceolantus* was most effective against *Pseudomonas aeruginosa* and *Bacillus cereus*. *Casurina equisitifolia* was effective against all most all organisms except *Salmonella typhi* and *Pseudomonas aeruginosa*. *Catharanthus roseus* was effective against both Gram-positive and Gram-negative organisms except *Salmonella typhi*, *Pseudomonas aeruginosa*,

Staphylococcus aureus, *Staphylococcus epidermis* and *Bacillus cereus*. *Grevellia robusta* showed activity against Gram-negative and Gram-positive organisms except *Salmonella typhi*, *Proteus vulgaris*, *Staphylococcus aureus* and *Staphylococcus epidermis*. *Kalanchoe pinnata* inhibited all except *Staphylococcus aureus*, *Staphylococcus epidermis* and *Bacillus megaterium*.

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