International Journal of Phytomedicine 3 (2011) 549-556

http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Studies on Antibacterial Activity of Methanolic Plant Extracts

Neeraj[1], Madhvi[1], Anand[1]

***Corresponding author:**

Neeraj Tandan

[1]Department of Biotechnology
Singhania University Rajasthan

A B S T R A C T

The antimicrobial activity of methanolic extracts was screened against 3 gram positive and 3 gram negative bacteria using agar well diffusion method. 80 µl of test extract was suspended in the wells. A control well is loaded with equal amount of the solvent i.e. methanol. The plates were then incubated at 37ºC for 24-48 hours. After proper incubation the plates around the extract impregnated wells and this clear zone of growth inhibition was measured in terms of diameter (mm). The inhibition zone is the mean of the three replicates, excluding the well diameter. The (-) sign indicates no activity. Only the aqueous extracts of A. nilotica (leaf and stem) and L. inermis (leaves) were found inhibitory to some of the organisms tested i.e. Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiela pneumoniae and Salmonella typhimurium. Salmonella typhimurium was only inhibited by aqueous extracts of L. inermis (leaves) whereas the rest of the aqueous extracts were found to be inactive against all the test organisms. The maximum activity was shown by aqueous extracts of L. inermis (leaf) against Staphylococcus aureus that is 1.9 mm diameter of inhibition zone and the minimum was observed in the case of aqueous extracts of Pergularia daemia (stem) i.e. 0.6 mm diameter of inhibition zone against Bacillus subtilis . Salmonella epidermidis was found to most resistance to aqueous extracts of the plant followed by Salmonella typhimurium.

Keyword- antimicrobial activity, methanolic extracts.

Introduction

Nature has been a source of medicinal agent for thousands of years and impressive number of modern drugs has been isolated from them. The wide spread use of herbal remedies has been documented in ancient texts, The Vedas and The Bible. The medicinal properties of the plants owes to the presence of secondary metabolites like terpenoid, alkaloid, flavanoid, phenol etc. present in them. According to estimate of WHO (World Health Organization) 80% of the world population rely on medicine obtained from the plants for their primary health care needs (Santos et al. 1995). India is endowed with a rich wealth of medicinal plants that make the good contribution to the development ancient India materia medica; one of the earliest treaties on Indian medicine the charka samhita (1000 B.C.) records the use of over 340 drugs of vegetable origin.

This revival of interest in plant derived drug is mainly due to the current widespread belief that green medicine is safe and more dependable compared the

costly synthetic drugs many of which have adverse side effects.

Scientific experiments on the antimicrobial properties of plant components were first documented in late 19th century. Since then a tremendous amount of work had been carried out worldwide that include screening of various medicinal plants used by traditional healers, for their antimicrobial activity and purification as well as characterization of the active substance. Till date a lot of work had been done in this area and a lot of it is yet to be revealed.

Materials and methods

To study the antibacterial effect of some medicinal plants against few bacterial and fungal pathogens.

All the bacterial cultures used in present study were obtained in a freeze-dried form from Microbial Type Culture Collection, IMTEC Chandigarh. The cultures are listed below:

1. Bacillus subtilis **(BS)** MTCC No. 121
2. Staphylococcus aureus **(SA)** MTCC No. 435
3. Staphylococcus epidermidis **(SE)** MTCC No. 1430
4. Escherichia coli **(EC)** MTCC No. 1610
5. Klebsiela pneumoniae **(KP)** MTCC No. 661
6. Salmonella typhimurium **(ST)** MTCC No. 98

Plant material collection:

Fresh plant material, collected from different locations, was washed thoroughly under running tap water and then oven dried at 50°C overnight. The dried material was then grinded into fine powder and stored in dark bottles.

Ten grams of air dried powder was placed in 100 ml of organic solvent (methanol) in a conical flask, plugged with cotton and then kept on a rotatory shaker at 190-220 rpm for 24 hours. After 24 hours it was filtered through 8 layers of muslin cloth and centrifuged at 5000g for 15 minutes. The supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original.

The bacterial cultures were grown in Nutrient broth at 37° C until the optical density reaches the absorbance of Mc. Farland No. 5 standard i.e. approximately 0.132 at 600 nm. At this absorbance a concentration of 10^8cells/ml was obtained. This suspension was used as inoculum for antimicrobial susceptibility testing.

Five antimicrobial agents namely Tetracycline, Chloramphenicol, Livofloxacin, Amoxicillin and Ofloxacin were tested for their effect on the test bacteria at various concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg/l). The tests were performed using agar well diffusion method with water as control. The wells were loaded with 60 µl of the antimicrobial agents.

The present study consists of evaluating different plants for their antibacterial properties. Sixteen plants were collected from different locations in Meerut and various parts of these plants like leaf, fruit, stem and flower were evaluated for their antibacterial potential.

Total 19 extracts were prepared in methanol. All the extracts were numbered as shown in the **Table 1**:

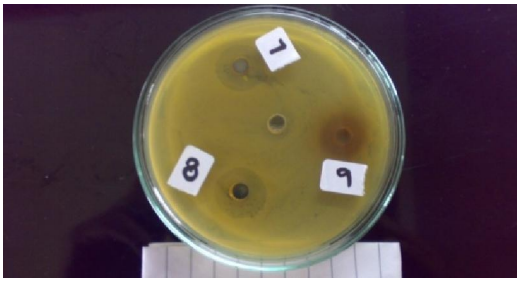
The antimicrobial activity of methanolic extracts was screened against 3 gram positive and 3 gram negative bacteria using agar well diffusion method. Agar plates were prepared and inoculated with the microbial suspension and then agar was punched to form wells of 5mm diameter. 80 µl of test extract was suspended in the wells. A control well is loaded with equal amount of the solvent i.e. methanol. The plates were then incubated at 37°C for 24-48 hours. After proper incubation the plates around the extract impregnated wells and this clear zone of growth inhibition was measured in terms of diameter (mm). The antimicrobial activity of the extracts was calculated in terms of zone of inhibition around the wells loaded with the test tube extracts.

Table 1: Numbers assigned to different extracts obtained from various plant parts extracted in three different solvents.

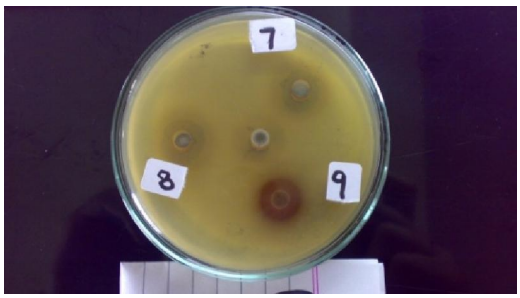
S. No.	Plant	Parts used	Methanolic extract no.
1.	Pomegranate	Epicarp	1
2.	Sambal	Sepals	4
		Petals	5
3.	Babool	Leaf	8
4.	Aakha	Leaf	12
5.	Arandi	Leaf	14
6.	Ashoka	Leaf	16
7.	Arjun	Leaf	17
8.	Neem	Leaf	18
9.	Pudina	Leaf	19
10.	Sadabahar	Leaf	20
11.	Tulsi	Leaf	21
12.	Tobacco	Leaf	23
13.	Orange	Epicarp	2
14.	Mehndi	Leaf	9
15.	Jangli Mirchi	leaf	11
		Stem	10
16.	Aksand	leaf	6
		Stem	7

Result and Discussion

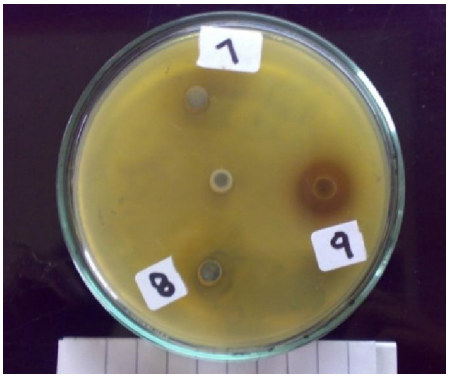
The results of antimicrobial activity of methanolic extracts have been depicted in **Table 2.** The inhibition zone is the mean of the three replicates, excluding the well diameter. The (-) sign indicates no activity. Only the aqueous extracts of A. nilotica (leaf and stem) and L. inermis (leaves) were found inhibitory to some of the organisms tested i.e. Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiela pneumoniae and Salmonella typhimurium. Salmonella typhimurium was only inhibited by aqueous extracts of L. inermis (leaves) the rest of the aqueous extracts were found to be inactive against all the test organisms. The maximum activity was shown by aqueous extracts of L. inermis (leaf) against Staphylococcus aureus **(Fig. 1.1A)** that is 1.9 mm diameter of inhibition zone and the minimum was observed in the case of aqueous extracts of Pergularia daemia (stem) i.e. 0.6 mm diameter of inhibition zone against Bacillus subtilis **(Fig 1.1C).** Salmonella epidermidis was found to most resistance to aqueous extracts of the plant followed by Salmonella typhimurium.



1A

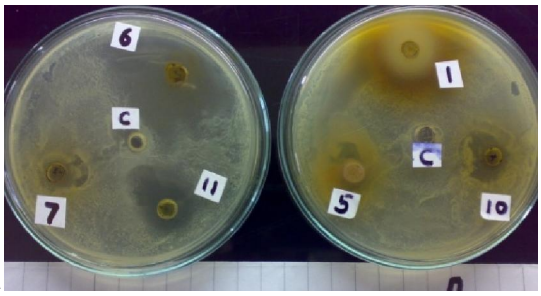


1B

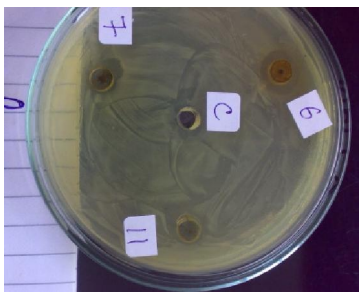


1C

Figure 1- Plates showing zone of inhibition by different aqueous plant extracts on: 1A- Staphylococcus aureus, 1B- Escherchia coli, and 1C- Bacillus subtilisMethanolic extracts of Punica granatum (leaf), Pergularia daemia (stem), Acacia nilotica (leaf, stem), Calotropis procera (Leaf, sepals), Ricinus communis (stem, leaf), Citrus aurantium (peels), Terminalia arjuna (leaf), Azadirachta indica (leaf), Mentha piperita (leaf), Ocimum (leaf), Catharanthus rescue(leaf), Lawsonia inermis (leaf) and Nicotiana rustica (leaf) do not show any inhibitory activity against the test organism. The maximum inhibitory activity to be in the case of Toddalia aculeata (leaf) i.e. 2.5 mm diameter against Staphylococcus aureus (Fig.2A) and minimum inhibition was observed in the case of Pergularia daemia (leaf) (0.7mm) diameter against Klebsiela pneumoniae (Fig.2B) was found to be most resistant followed by E. coli. The rest of the test organisms were more or less equally susceptible.



2A



2B

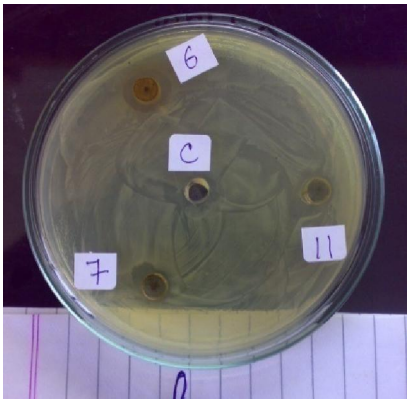
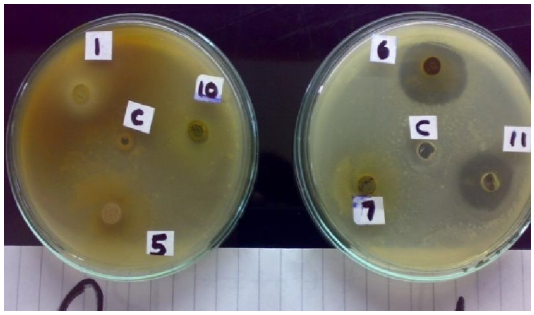
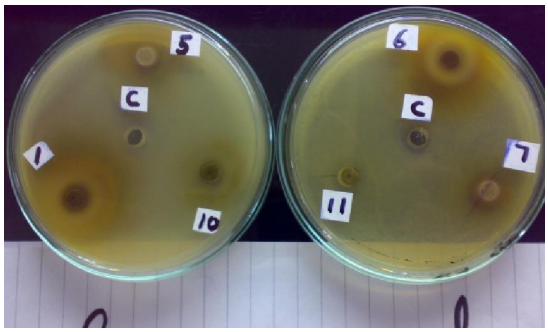
2C 

Figure 2- Plates showing zone of inhibition by different methanolic plant extracts on: 2A- Staphylococcus aureus, 2B- Klebsiela pneumoniae, and 2C- Escherichia coli.

3A 

3B 

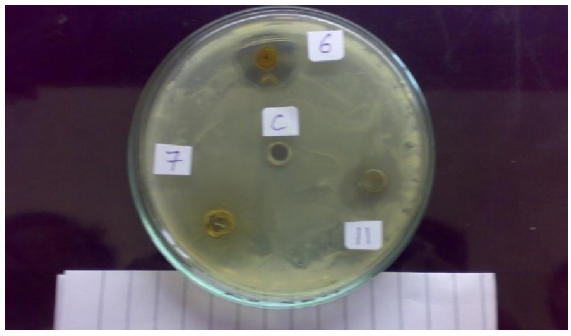
3C 

Figure 3- Plates showing zone of inhibition by different methanolic plant extracts on: 3A- Bacillus subtilis, 3B- Salmonella typhimurium, and 3C- Staphylococcus epidermidis.

Analyzing the data it was observed that plant extract inhibited the gram positive microorganisms better than gram negative microorganisms. The result is in agreement with previous report that plant extract are active against gram positive bacteria than gram negative bacteria [1].The result can be explained that the outer membrane of gram positive bacteria known to present a barrier to the penetration of numerous antibiotic molecule, and the periplasmic space contains enzymes that are able to break down foreign molecules introduced from outside [2-4] and efflux that reduce the cellular level of antibiotics [5].The broad spectrum of antibacterial activity found in the study may be attributed to the presence of secondary metabolite of various chemical types present in the plant. Different plants possess different constituents and in different concentration which account for different antimicrobial effect as also suggested earlier [6].

Table 2: Antimicrobial activity of Methanolic and Aqueous extracts of the screened medicinal plants against different microbial strains.

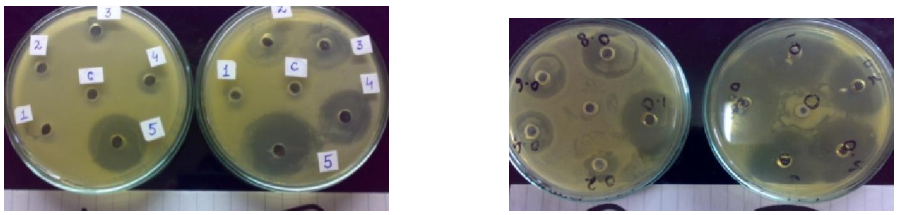
Name of plant	Plant part	Zone of inhibition (mm)					
		BS	SA	SE	EC	KP	ST
		Met	Met	Met	Met	Met	Met
Anar	Leaf	-	-	-	-	-	0.9
	Epicarp	-	-	-	-	-	-
Sambal	Sepal	1.7	-	1.6	1.9	-	-
	Petals	-	-	2.0	-	-	-
Babool	Leaf	-	-	-	-	-	-
	Stem	-	-	-	-	-	-
Aakha	Leaf	2.0	-	1.2	-	-	-
	Stem	-	-	-	-	-	-
Arahandi	Stem	-	-	-	-	-	-
	Leaf	-	-	-	-	-	-
Ashoka	Leaf	-	1.9	-	1.2	-	-
Arjun	Leaf	-	-	-	-	-	-
Pudina	Leaf	-	-	-	-	-	-
Sadabahar	Leaf	-	-	-	-	-	-
Tulsi	Leaf	-	-	-	-	-	-
Tobacco	Leaf	-	-	-	-	-	-
Orange	Epicarp	-	-	-	-	-	-
Mehandi	Leaf	-	-	-	-	-	-
Jangli Mirchi	Leaf	1.2	2.2	-	-	-	1.0
	Stem	-	1.0	-	-	-	-
Aksand	Leaf	1.8	-	-	-	0.7	1.5
	Stem	-	-	-	-	-	-
Neem	Leaf	-	-	-	-	-	-

Bacillus subtilis **(BS);** Staphylococcus aureus **(SA);** Staphylococcus epidermidis **(SE);** Escherichia coli **(EC);** Klebsiela pneumoniae **(KP);** Salmonella typhimurium **(ST).** Zone of inhibition (mm) excluding well diameter. (–) sign indicates no activity.

As observed by comparing the **Table 2,** it was clearly indicated that methanolic extracts are more potent anti bacterial agents. Successful prediction of botanical compounds from plant material is largely dependent on the type of solvents used in the extraction procedure. Traditional healers use primarily water as solvent but in this study, plant extracts in organic solvent (methanol) provided more consistent antimicrobial activity. The observation can be rationalized in terms of the polarity of compounds being extracted by each solvent and, in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in different media used in the assay. The result obtained in accordance to the preexisting literature [6-8].

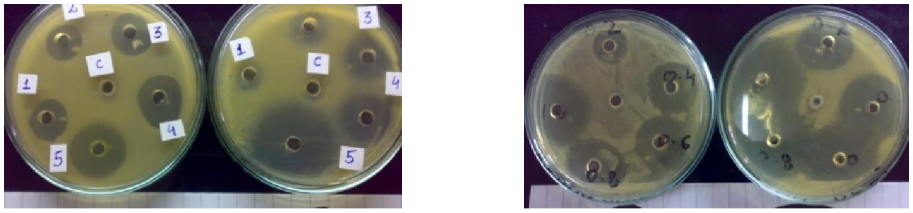
Effect of Antibiotics On The Growth Of Bacteria

Five antibiotics namely Amoxicillin, Tetracycline, Chloramphenicol, Levofloxin and Ofloxacin were used to access the susceptibility of test bacteria/organism for them. All the test organisms were observed to be susceptible to four of the antibiotics used (Amoxicillin, Tetracycline, Chloramphenicol, Levofloxin) except Staphylococcus epidermidis (resistant to Tetracycline and Amoxicillin) and Kleibsella pneumoniae (susceptible to only high concentration of Tetracycline and Amoxicillin; **(Fig 4.).** All the strains were resistant to Ofloxacin.



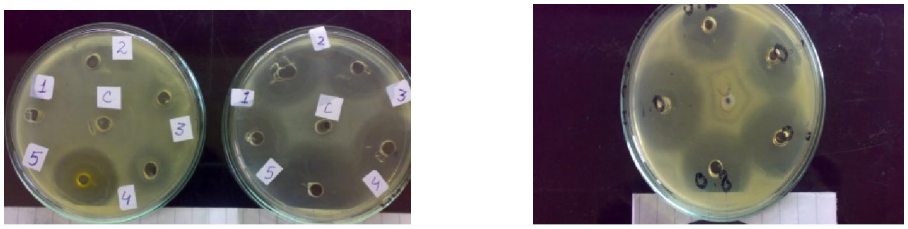
(a) (b) (c) (d)

Fig. 4A



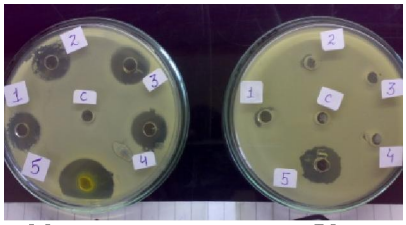
(a) (b) (c) (d)

Fig. 4B



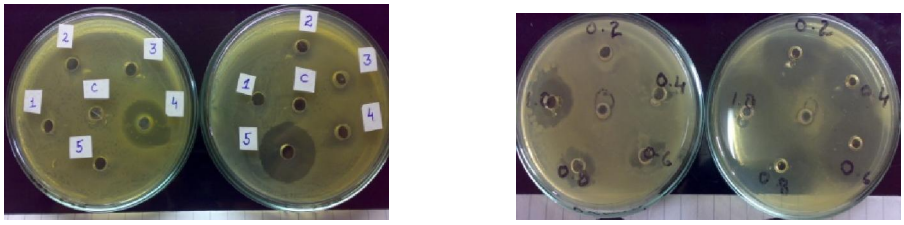
(a) (b) (d)

Fig. 4C



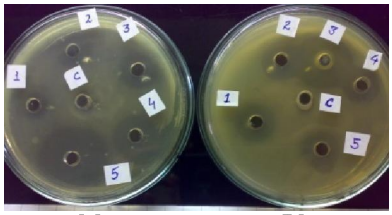
(a) (b)

Fig. 4D



(a) (b) (c) (d)

Fig. 4E



(a) (b)

Fig.4F

Figure 4- Plates showing zone of inhibition by different antibiotics on:
4A Bacillus subtilis, 4B Staphylococcus aureus, 4C- Staphylococcus epidermidis, 4D Escherichia coli, 4E- Salmonella typhimurium and 4F- Klebsiela pneumonia
(a): Tetracyclin; (b): Chloramphenicol; (c): Amoxicillin; (d): Levofloxacin.

All the active extracts were found to be stable at room temperature up to three months and did not show any reduction of activity against the sensitive bacteria as compared to the activities of first day.

References

1. Vlietinck AJ, Van Hoff L, Totte J, Lasure A, Vanden Berghe, Roreangobo PC and Mvukeyuniwami J. Ethnopharmacol J. 1995;46;31.
2. Poole K. Bacterial multidrug resistance emphasis on efflux mechanisms in Pseudomonas aeruginosa. J. Antimicrob. chemother. 1994;34:453-456.
3. Schaechter M, Engleberg NC, Eisentein BI, Medoff G. Mechanisms of microbial disease. 3rd ed. Philadelphia, Lippincott, Williams & Wilkins Silver L. L. (1993) Antimicrobial agents. Chemother. 1999;37;377.
4. Duffy CF and Power RF. .Antioxidant and antimicrobial properties of some Chinese plant extracts. Int. J. Antimicrobial agents. 2001;17:527-529.
5. Nikaido H. Outer membrane barrier as a mechanism of antimicrobial resistance. Antimicrob. Agents chemother. 1989;33:1831-1836.
6. Parekh J, Nair R and Chanda S. Preliminary screening of some folkloric plants from western India for potential antimicrobial activity. Indian J pharmacol (India). Perez P 2005.
7. Nair R and Chanda S. Anticandidal activity of Punica granatum exhibited in different solvents. Pharma. Biol. 2005;43;21-25.
8. Santos PRV, Oliveira ACX, Tomassini TCB. Controle microbiogico de prudotos fitoterapicos. Rev. Farm. Bioquim. 1995;31;35-38.