INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

ANTIBACTERIAL EFFECTS OF PLANTS EXTRACTS ON HUMAN MICROBIAL PATHOGENS & MICROBIAL LIMIT TESTS

Rajendra Sapkota, Rubin Dasgupta*, Nancy and DS Rawat

Department of Microbiology and Biotechnology, Baba Farid Institute of Technology, Dehradun, India.

ABSTRACT

While 25-50% of current pharmaceuticals are derived from plants, none are used as antimicrobials. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids which have been found in-vitro to have antimicrobial properties. The objective of this project work was to evaluate the antibacterial effects of plants extract on human microbial pathogens. Antibacterial effects of plants extract are to determine the ability of plant part extract to kill or inhibit the growth of living bacterial cells. For this, the method generally used is Cylinder Plate Method or Cup Plate Method**.** The cylinder plate method is based upon diffusion of the plant extract from a vertical cylinder through a Solidify Agar layer in a petridish to an extent such that growth of the added microorganism is prevented entirely in a zone around the cylinder containing a extract of the plant. Guava leaves with mid ribs (MR) and without midribs (WMR) tested against *B.subtilis, E.coli, P.aeruginosa, S.abony*. The inhibition zone surrounding the wells varied from 15.23 – 20.52 mm with all bacteria being sensitive to Guava leaves extract. Lemon leaves (L) & juice (J) tested against *B.subtilis, E.coli, P.aeruginosa, S.abony S.aureus* The inhibition zone surrounding the wells varied from 18.91 – 20.44 mm. Garlic cloves tested against *B.subtilis, E.coli, P.aeruginosa, S.abony S.aureus* The inhibition zone surrounding the wells varied from 16.82 – 20.22 mm. Neem Bark heat treated (HT) and without heat treatment (WHT) tested against *B.subtilis, E.coli, P.aeruginosa, S.abony S.aureus* The inhibition zone surrounding the wells varied from 12.74 – 14.95 mm (B-HT) & 12.92 – 16.64 mm. Ginger stem tested against *B.subtilis, P.aeruginosa, S.aureus.* The inhibition zone surrounding the well is observed only against S.aureus. No antibacterial activity (na) was shown towards *B.subtilis & P.aeruginosa.*

Keywords: *B.subtilis, E.coli, P.aeruginosa, S.abony, S.aureus,* antibacterial, plant extracts.

INTRODUCTION

Bacteria are responsible for high mortality rates in numerous developing countries with as many as 50,000 people dying daily as a consequence of infections. Plants remedies are increasingly being recognized by scientists as a very important low cost alternative to industrially produced antibiotics which are not available to all who need them because of their high price. So present project work express that plants have great potential as antimicrobial compounds against microorganisms. Thus they can be used in the treatment of infectious diseases caused by pathogenic bacteria. Microbial Pathogens such as *E.coli, S.aureus, S.abony, P.aeruginosa, B.subtilis* are widely distributed in nature causing considerable mortality and morbidity in the population.

It has been reported that there are more than 1.3 billion cases of human Salmonellosis annually with 3 million deaths worldwide. *E.coli* has been isolated from various environments and is reported to cause several deaths. *Staphylococcus aureus* causes a variety of suppurative (pus-forming) infections and toxinoses and more serious infections such as **pneumonia**, **mastitis**, **meningitis**, and

urinary tract infections. *S. aureus* is a major cause of **hospital acquired (nosocomial) infection** of surgical wounds and infections associated with indwelling medical devices. *E.coli, B.subtilis* are well known to causes **food poisoning**.

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkanoids and flavonoids which have been found in-vitro to have antimicrobial properties. The extract of different parts of following plants have been used in the present project work to check their antibacterial effectiveness against human bacterial pathogens.

1. *Psidium sp.*
2. *Allium sativum*
3. *Citrus limon*
4. *Azadirakhta indica*
5. *Zingiber officinale*

The antimicrobial properties of these plants are as under –

1. ***Psidium sp.***- Guava [leaves] have several chemical constituents such as comarins, essential oils, flavonoids, triterpenes and ellagitannins which are known to have antimicrobial properties.
2. ***Allium sativum*** – Garlic [Cloves] When crushed, *Allium sativum* yields allicin, a powerful antibiotic and antifungal compound (phytoncide).Allicin has a variety of antimicrobial activities. Allicin in its pure form was found to exhibit i) antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, including multidrug-resistant enterotoxicogenic strains of *Escherichia coli*; ii) antifungal activity, particularly against *Candida albicans*; iii) antiparasitic activity, including some major human intestinal protozoan parasites such as *Entamoeba histolytica* and *Giardia lamblia* and iv) antiviral activity. The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, e.g. alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase, which can affect essential metabolism of microorganisms.
3. ***Citrus limon***- Lemon juice is about 5% (approximately 0.3 mole per liter) citric acid, which gives lemons a tart taste, and a pH of 2 to 3. Antibacterial uses because it has a low pH.
4. ***Azadirakhta indica***- Neem [Bark] diterpenoids, margolone and isomargolonone isolated from neem stem bark are active against *Klebsiella*, *Staphylococcus* and *Serratia*. : Oil from the leaves, seeds and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, including *M. tuberculosis* and streptomycinresistant strains. *In vitro*, it inhibits *Vibrio cholerae*, *Klebsiella pneumoniae, M. tuberculosis* and *M. pyogenes*. Antimicrobial effects of neem extract have been demonstrated against *Streptococcus mutans* and *S. faecalis*.
5. ***Zingiber officinale***- Ginger[Stem] have a mixture of zingerone, shogaols and zingerols, volatile oils that compose about one to three percent of the weight of fresh ginger. These have been antibacterial in nature. Ginger compounds are active against a form of diarrhea which is the leading cause of infant death in developing countries. Zingerone is likely to be the active constituent against enterotoxigenic *Escherichia coli* heat-labile enterotoxin-induced diarrhea.

MATERIALS AND METHODS

Bacterial Cultures -
Staphylococcus aureus
Salmonella abony
Escherichia coli
Bacillus subtilis
Pseudomonas aeruginosa

Culture Media - Soyabean Casein Digest Agar

Plants Extract -
Psidium sp.(Guava)
Azadirakhta indica (Neem)
Allium sativum (Garlic)
Zingiber officinale (Ginger)
Citrus limon (lemon)

Other requirements for Extract preparation - plant part samples, knife, mortar and pestle, muslin cloth, funnel, Raw water, purified water, Sterile water, test tubes for extract as per requirement.

Test Requirements - sterile saline tubes (5, each of 5ml), 70% IPA, sterile test tubes (15), sterile petriplates (15), 250ml conical flasks (5), Borer, Micropipette, sterile tips, sterile inoculating loop. Autoclave, Shaker, Oven (32°C-35°C), Laminar Air Flow, Incubator, Weighing Balance, Vernier Caliper

METHOD USED- AGAR DIFFUSION OR CUP PLATE METHOD

Loopful of the cultures of *Staphylococcus aureus, Salmonella abony, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* from old refrigerated SCDA slants were

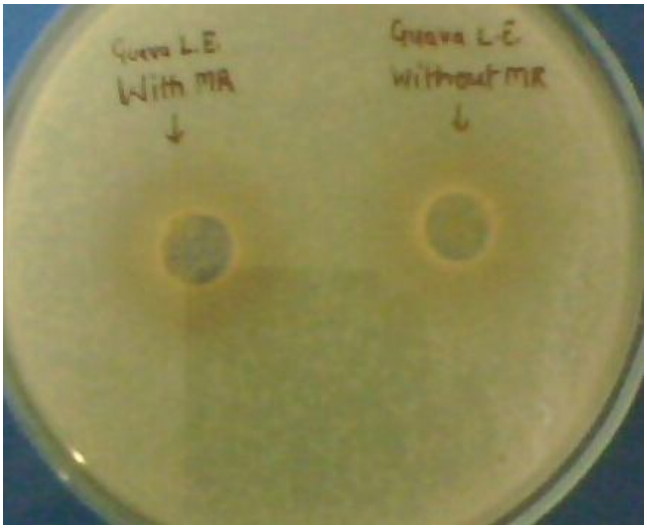
inoculated by streaking on 24 hrs old preincubated fresh SCDA slants. After inoculation these slants were incubated at 32°C for 24- 48 hrs. Old culture is transferred separately in 5 ml sterile saline solution tube to prepare suspension of each culture. These 5 ml suspension of each culture are transferred separately to 150 ml sterile SCDA when cooled to a temperature of 40 - 45°C. Each conical flask having SCDA and culture suspension is shaken to allow uniform distribution of microbial cells in medium. After shaking each SCDA medium with culture suspension is poured in three plates. Labelled 15 petriplates (three for each bacterial culture) after pouring are allowed to solidify. After solidification of the medium in plates, the wells are cut in each plate using sterile borer. The amount of 0.1 ml of 10% (w/v) extract of different plants to be tested were poured into different wells. The plates were incubated at 32°C for 24 hrs. After incubation the plates were observed for the presence of zone of inhibition. If present, the diameter of zone of inhibition was measured with the help of vernier calliper.

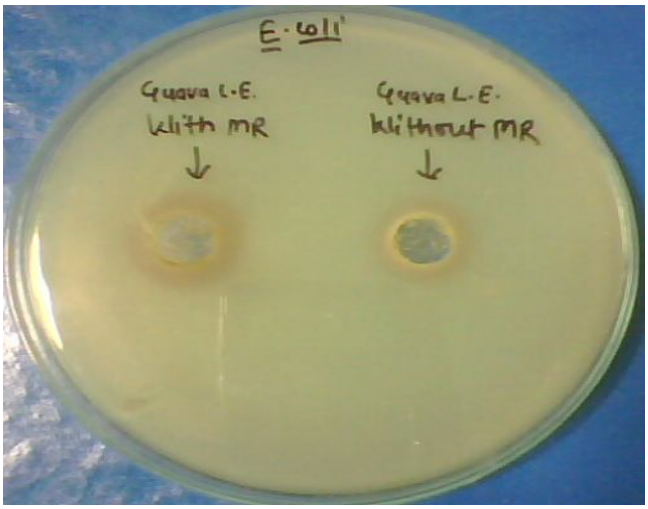
RESULTS AND DISCUSSION

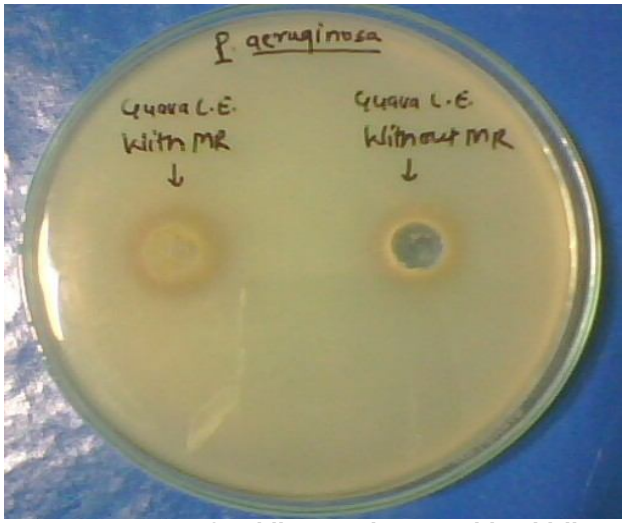
***Psidium sp.(*Guava)**

Table shows the Diameter of zone of inhibition for the extract of Guava leaves with mid ribs (MR) and without midribs (WMR) tested against *B.subtilis, E.coli, P.aeruginosa, S.abony*. The inhibition zone surrounding the wells varied from 15.23 – 20.52 mm with all bacteria being sensitive to Guava leaves extract.

S. No.	Bacterial Culture	Leaf Ext.	Diameter of Zone of Inhibition (mm)			
			P-1	P-2	P-3	Mean
1.	*B. subtilis*	MR	20.50	20.48	20.57	20.5166
		WMR	18.54	18.72	19.47	18.91
2.	*E.coli*	MR	18.47	18.31	17.77	18.1833
		WMR	15.97	15.64	14.08	15.23
3.	*P.aeruginosa*	MR	16.96	16.77	16.18	16.6366
		WMR	15.77	15.60	15.02	15.4633
4.	*S.abony*	MR	18.78	18.90	18.66	18.76
		WMR	18.09	18.21	17.59	17.9633







MR – Extract of *Psidium sp.* leaves with Midribs
WMR – Extract of *Psidium sp.* leaves without Midribs

Citrus limon **(Lemon)**

Table shows the Diameter of zone of inhibition for the extract of Lemon leaves (L) & juice (J) tested against *B.subtilis, E.coli, P.aeruginosa, S.abony S.aureus* The inhibition zone surrounding the wells varied from 18.91 – 20.44 mm with all bacteria being sensitive to Lemon juice. On the other hand lemon leaves extract has shown antibacterial effect against *B.subtilis, E.coli* & *S.aureus and* no activity (na) for *P. aeruginosa* & *S.abony.*

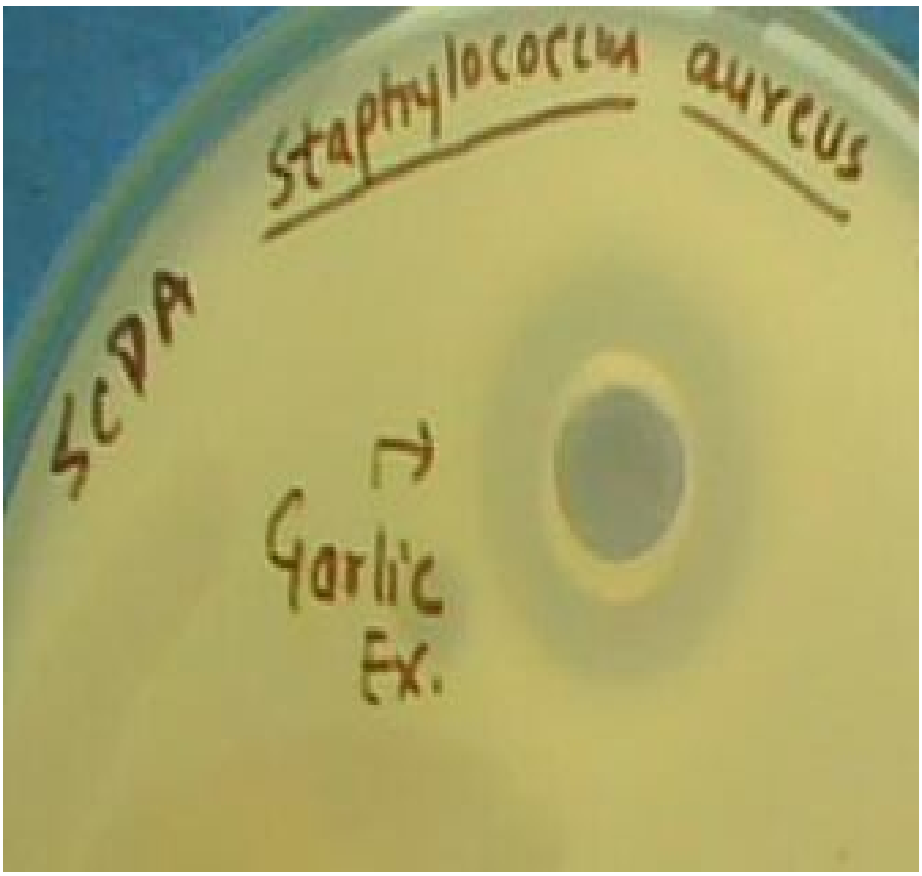
S. No.	Bacterial Culture	Extract	Diameter of Zone of Inhibition (mm)			
			P-1	P-2	P-3	Mean
1.	*B.subtilis*	L	24.44	20.02	17.51	20.3233
		J	19.87	19.16	18.33	19.12
2.	*E.coli*	L	21.00	18.71	22.12	20.61
		J	19.15	20.43	21.11	20.23
3.	*P.aeruginosa*	L	na	na	na	na
		J	21.34	19.70	20.28	20.44
4.	*S.abony*	L	na	na	na	na
		J	18.78	19.49	18.47	18.9133
5.	*S.aureus*	L	21.40	19.28	18.51	19.73
		J	22.38	19.54	17.91	19.9433

Allium sativum **(Garlic)**

Table shows the Diameter of zone of inhibition for the extract of Garlic cloves tested against *B.subtilis, E.coli, P.aeruginosa, S.abony S.aureus* The inhibition zone surrounding the wells varied from 16.82 – 20.22 mm with all bacteria being sensitive to Guava leaves extract.

S. No.	Bacterial Cultures	Diameter of Zone of Inhibition (mm)			
		P-1	P-2	P-3	Mean
1.	*B.subtilis*	17.53	17.40	18.06	17.6633
2.	*E.coli*	18.90	20.05	19.47	19.4733
3.	*P.aeruginosa*	17.05	16.61	18.17	17.2766
4.	*S.abony*	17.10	16.87	16.49	16.82
5.	*S.aureus*	19.68	20.23	20.75	20.22


Pseudomonas aeruginosa
SCDA
Citrus →


Staphylococcus aureus
SCDA
→
Garlic
Ex.

***Azadirakhta indica* (Neem)**

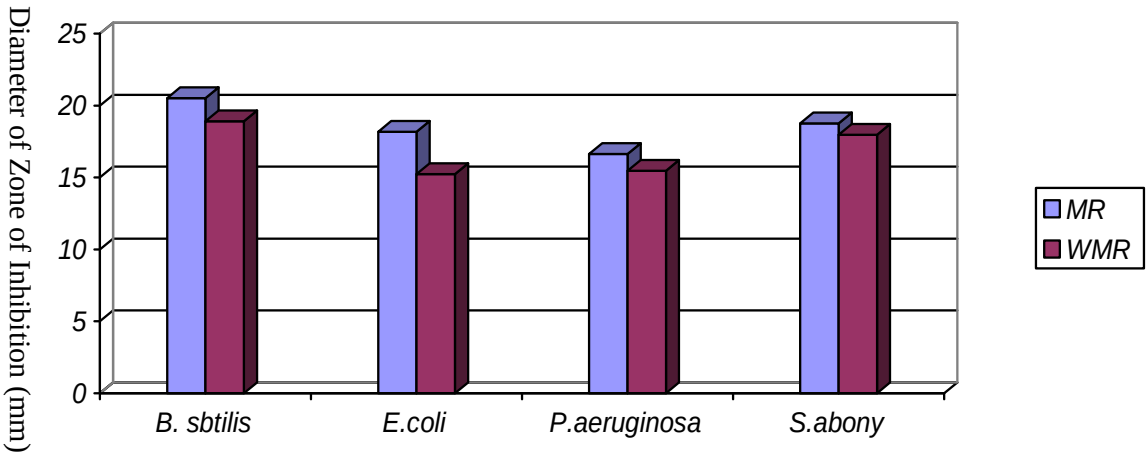
Table shows the Diameter of zone of inhibition for the extract of Neem Bark heat treated (HT) and without heat treatment (WHT) tested against *B.subtilis, E.coli, P.aeruginosa, S.abony S.aureus* The inhibition zone surrounding the wells varied from 12.74 – 14.95 mm (B-HT) & 12.92 – 16.64 mm (WHT) with all bacteria being sensitive to Neem Bark Extract.

S. No.	Bacterial Cutures	Bark Extract	Diameter of Zone of Inhibition (mm)			
			P-1	P-2	P-3	Mean
1.	*B.subtilis*	B-HT	13.62	12.04	13.84	13.1666
		WHT	14.07	14.28	15.23	14.5266
2.	*E.coli*	B-HT	12.75	12.37	13.30	12.8066
		WHT	13.15	12.27	13.36	12.9266
3.	*P.aeruginosa*	B-HT	14.96	14.42	15.48	14.9566
		WHT	16.31	16.01	15.85	16.0566
4.	*S.abony*	B-HT	13.44	12.49	12.29	12.74
		WHT	13.46	13.48	12.41	13.1166
5.	*S.aureus*	B-HT	14.73	14.76	14.77	14.7533
		WHT	15.53	16.94	17.46	16.6433

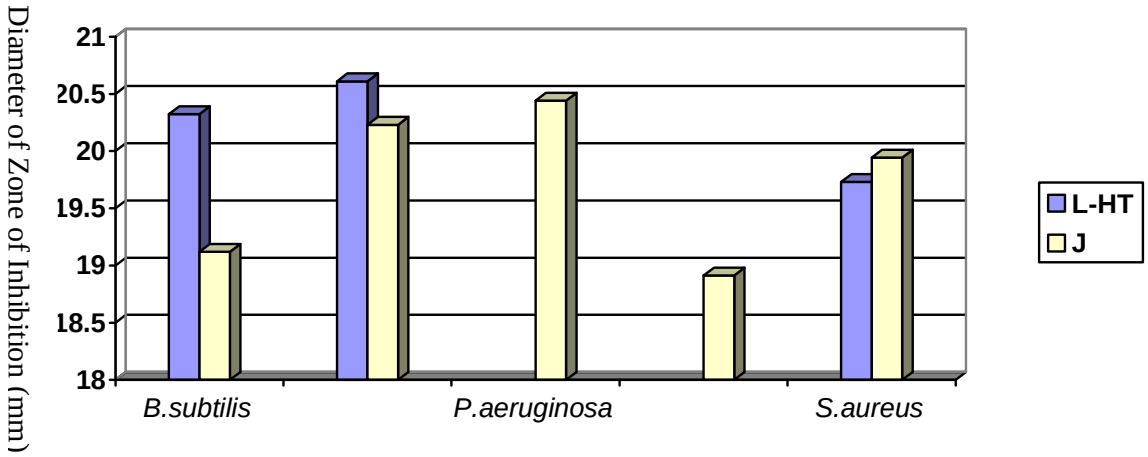
***Zingiber officinale* (Ginger)**

Table shows the Diameter of zone of inhibition for the extract of Ginger stem tested against *B.subtilis, P.aeruginosa, S.aureus.* The inhibition zone surrounding the well is observed only against S.aureus. No antibacterial activity (na) was shown towards *B.subtilis & P.aeruginosa.*

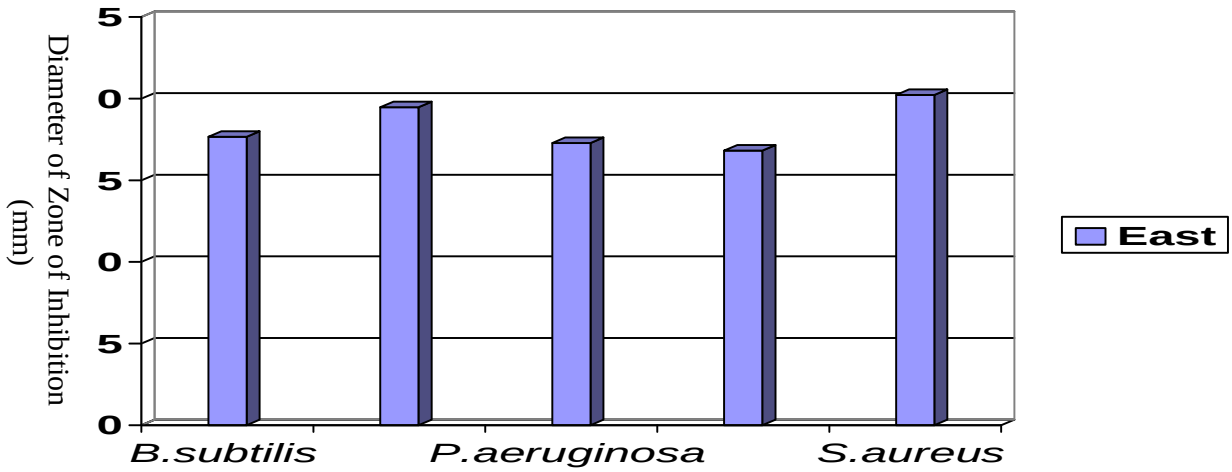
S. No.	Bacterial Culture	Diameter of Zone of Inhibition (mm)			
		P-1	P-2	P-3	Mean
1.	*S.aureus*	20.97	20.95	19.62	20.5133
2.	*B.subtilis*	na	na	na	Na
3.	*P.aeruginosa*	na	na	na	Na



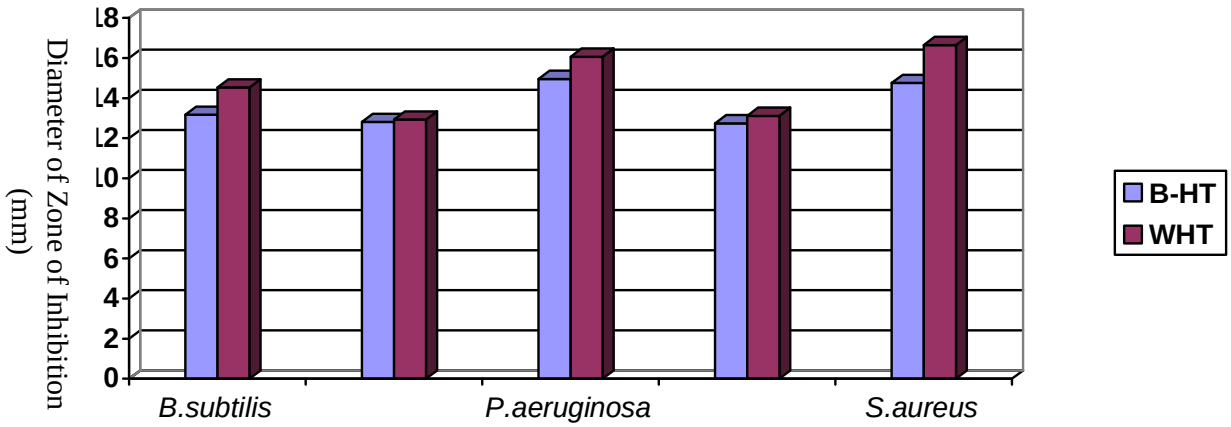
Psidium sp. **(Guava)**
B.subtilis > S.abony > E.coli > P.aeruginosa
Guava leaves extract has shown maximum antimicrobial activity towards *B.subtilis.*



Citrus limon **(Lemon)**
E.coli > B.subtilis > S.aureus **(L)**
P.aeruginosa > E.coli > S.aureus > B.subtilis > S.abony **(J)**
Lemon leaves extract (L) and juice (J) has shown maximum antibacterial activity towards *E.coli & P.aeruginosa* respectively.



***Allium sativum* (Garlic)**
S.aureus > E.coli > B.subtilis > P.aeruginosa > S.abony
Garlic cloves extract has shown maximum antimicrobial activity towards *S.aureus.*



***Azadirakhta indica* (Neem)**
***P.aeruginosa* > *S.aureus* > *B.subtilis* > *E.coli* > *S.abony* (B-HT)**
***S.aureus* > *P.aeruginosa* > *B.subtilis* > *S.abony* > *E.coli* (WHT)**
Neem bark extract heat treated (HT) & without heat treatment (WHT) has shown maximum antimicrobial activity towards *P.aeruginosa* & *S.aureus* respectively.

SUMMARY AND CONCLUSION

Parts of several plants are used as medicines against gastroenteritis, diarrhoea, skin and many other infectious diseases by those who cannot afford or don't have access to antibiotics. The present study screened the antibacterial effects of aqueous extracts of Guava, Lemon leaves, Neem bark, Garlic cloves, Ginger stem & Lemon juice. These extracts were treated against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Salmonella abony*. Guava leaves inhibited the growth of *B.subtilis* to maximum extent. Both Neem bark & Garlic cloves inhibited the growth of *S.aureus* to greater extent. On the other hand Lemon juice and leaves extract inhibited the growth of *P.aeruginosa* and *E.coli* respectively

to high level. These data support the use of such plants based medicines in treatment of infectious diseases where access to commercial antibiotics is restricted. In conclusion plants extracts even in aqueous form are active against human microbial pathogens thus making up potential sources of new antimicrobial compounds.

MICROBIOLOGICAL STANDARDS FOR MICROBIAL LIMIT TEST ENVIRONMENT MONITORING

1. **MICROBIAL LIMIT TEST**

Standards for Water Samples

Water Sample	Limit cfu /ml
Raw water	500 cfu
Purified water	100 cfu
WFI	NMT 10 cfu

2. ENVIRONMENTAL MONITORING

1. Settle Plate Technique

Area	Limit cfu/plate	Alert Limit	Action Limit
Class 100 (A)	Less than 1	1 cfu	1 cfu
Class 1000(B)	Less than 5	3 cfu	5 cfu
Class 10,000(C)	Less than 50	20 cfu	30 cfu
Class 1,00,000(D)	Less than 100	50 cfu	80 cfu

2. Active Air Sampling

Area	Limit cfu/m3	Alert Limit	Action Limit
Class 100 (A)	Less than 1	1 cfu	1 cfu
Class 1000(B)	Less than 10	3 cfu	5 cfu
Class 10,000(C)	Less than 100	20 cfu	50 cfu
Class 1,00,000(D)	Less than 200	50 cfu	80 cfu

3. RODAC Plate Technique

Area	Alert Limit	Action Limit
Class 100 (A)	2cfu including floor	3cfu inclu. floor
Class 10,000(C)	3 cfu (6 inclu. floor)	5 (10 inclu.floor)
Class 1,00,000(D)	30 cfu	50 cfu

Area	Gloves		Personnel clothing	
	Alert limit	Action Limit	Alert Limit	Action Limit
Class 100(A)	2 cfu	3 cfu	3 cfu	5 cfu
Class 1000(C)	6 cfu	10 cfu	12 cfu	20 cfu

Environment Monitoring Test results for different locations
By Active Air Sampling

Sr. No.	Location	Grade of area	No. of samples	TBC/1000lt.			TFC/1000lt.		
				1	2	3	1	2	3
1.	Filling Room	A	3	< 1	< 1	<1	Nil	Nil	Nil
2.	Media Prep. Room	D	3	22	27	32	Nil	Nil	Nil
3.	Washing Room	D	3	29	38	41	Nil	Nil	Nil

By Settle Plate Method

S. No.	Location	Grade of area	No. of samples	TBC/Plate		TFC/Plate	
				1	2	1	2
1.	LAF	A	1	< 1	< 1	Nil	Nil
2.	MLT Room	B	2	2	1	Nil	Nil
3.	Biosafety Room	B	2	Nil	1	Nil	Nil

REFERENCES

1. Dubey R.C, and D.K. Maheshwari. 2002. Practical Microbiology, S. Chand & Co. P. Ltd., Ram Nagar, New Delhi.
2. Practical Microbiology, 1st Edition by Dubey and Maheshwari, 2009.
3. Experiments in Microbiology, Plant Pathology and biotechnology, 4th edition by K.R. Aneja, 2009.