

## EVALUATION AND SCREENING OF ANTIMICROBIAL ACTIVITY OF SOME IMPORTANT MEDICINAL PLANTS OF ASSAM

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### ABSTRACT:

The evolution and spread of antibiotic resistance, as well as the evolution of new strains of disease causing agents, is of great concern to the global health community. Our ability to effectively treat diseases is dependent on the development of new pharmaceuticals, and one potential source of novel drugs is traditional medicine. Now-a-days, plants have been exploited as a powerful and potential source for medicinal drugs. Herbal drugs are mainly focussed as an alternative source against manifestations caused by various micro-organisms due to the increasing resistance of existing antimicrobial agents. The present study attempts to evaluate the antimicrobial efficacy of the crude methanol extract of *Ocimum gratissimum* (Local name: Ram tulashi), *Spondias pinnata* (Local name: Amora), *Terminalia chebula* (Local name: Silikha) and *Hedyotis pinifolia* (Local name: Bon jaluk) against six Gram-positive bacterial strains (*Bacillus subtilis*, *Enterococcus faecalis*, *Micrococcus luteus*, *Listeria innocua*, *Listeria monocytogenes* and *Staphylococcus aureus*) and three Gram-negative bacterial strains (*Pseudomonas aeruginosa*, *Enterobacter cloacae* and *Enterobacter aerogenes*) by agar well diffusion method. Methanol extract of all the plants under study were found to be effective against all the reference strains. Highest zone of inhibition was observed against *B. subtilis* (23mm of clear zone) followed by *L. innocua* (16 mm of clear zone) in case of *Terminalia chebula*. The findings indicated promising antibacterial activity of the crude extracts of the above plants and needs further exploration for their effective use in both modern and traditional system of medicines.

**Key word:** medicinal plants, antibacterial, agar well diffusion

### INTRODUCTION:

Developing countries like India, Brasil, China etc. have been using the traditional system of medicine from centuries for treating various ailments and diseases. Northern Eastern region of India especially Assam possess a rich source of bio-diversity and it is considered as a major hot spot of India. The wide geographical and climatic diversity of Assam provides a repository of valuable indigenous system of medicine, as their extracts in various forms are being used in traditional system of medicine for the treatment of human ailments. Now a day's plants have been exploited as a powerful and potential medicinal drug. Herbal drugs are mainly focussed as an alternative source against manifestations caused by various microorganisms due to the increasing resistance of existing antimicrobial agents. Studies by various researchers have proved that plants are one of the major sources for drug discovery and development (Rates SMK, 2001, Pasquale et al. 1984, Gordon et al. 2005). Plants are reported to have antimicrobial, anticancer, antiinflammatory, antidiabetic, hemolytic, antioxidant, larvicidal properties etc.

Antibiotic resistance has become a global concern in recent years especially in developing countries. As infectious diseases are one of the major causes of mortality in these countries, the screening of

higher plants for their natural products has been extensively focussed. This could lead to its successful use as a potential drug which confers to its therapeutic use. Till now only a small percentage of these plants have been explored so far and further phytochemical investigation and pharmacological screening are required for its successful use in ethnomedicine. Most of the medicinal plants contain active constituents which needs further screening for its use as antimicrobial substance. Studies reported that secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids that are present in these plants might confer to its antimicrobial activity (Gayathri Gunalan *et. al.*, 2011). Due to a rapid increase in the rate of antibiotic resistance in microorganisms and side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs and these plants are readily available in rural areas at relatively cheaper than modern medicine.

*Ocimum gratissimum L.* is known as tulsi, tulasi, vishnu priya, surasa in sanskrit, ram tulashi in assamese belongs to the family Lamiaceae. The plant is commonly used in folk medicine to treat different diseases, e.g. upper respiratory tract infections, diarrhea, headache, ophthalmic, skin diseases, pneumonia, and also as a treatment for cough, fever, and conjunctivitis (Corrêa 1932 & Onajobi 1986). Ethanol extracts of *O. gratissimum* have been reported to show more antibacterial activity against *S. aureus* than *E. coli* (Agatemor 2009).

*Terminalia chebula* (Family: Combretaceae, Haritaki, Harida, Harar in Hindi, Silikha in Assamese) called the "king of medicines" and is always listed first in the Ayurvedic materia medica because of its extraordinary power of healing. In Ayurveda it is considered to destroy all diseases and eliminate all waste from the body. This herb is widely used specially in India for the treatment of diseases like digestive diseases, urinary diseases, diabetes, skin diseases, parasitic infections, heart diseases, irregular fevers, flatulence, constipation, ulcers, vomiting, colic pain and haemorrhoids. *Terminalia Chebula* has a strong effect against the herpes simplex virus HSV, has antibacterial activity and exhibits strong cardio tonic properties. *Terminalia Chebula* also has antioxidant components which indicate it can increase the life of tissues.

*Spondias pinnata* belongs to family Anacardiaceae commonly known as Ambara in Hindi, Amora in Assamese. The bark is recommended for stomach ache, dysentery, rheumatism and swollen joints. A crude extract of *S. pinnata* has been reported to show antibacterial activity. In ethnomedicine, equal quantities of bark juice of *S. pinnata* and *Syzygium cumuni* are prescribed as a remedy for dysentery. An aqueous extract of this plant inhibits the citrus canker of lime (Bibhabasu Hazra *et al.* 2008).

*Hedyotis pinifolia* (Family: Rubiaceae) commonly known as Satwin, Saptaparni in Hindi, Ban jaluk in Assamese. The plant used as a traditional medicine in India for the treatment of Jaundice and other liver diseases. The leaves and roots are used as a lotion or poultice applied to the affected part of the body. The extracts are also taken as a tonic to treat various ailments.

Based on above information this experiment was designed to study the microbicidal activity of these herbs against nine bacterial strains responsible for community acquired infections. These findings could yield great significance in health delivery system, since it could be used as an alternative treatment to orthodox antibiotics in the treatment of diseases caused by the bacterial pathogens.

## **MATERIALS AND METHODS:**

### **Collection of Plant Material:**

For the preparation of plant extracts, samples of leaves of *Ocimum gratissimum* (Family: Lamiaceae); fruit of *Spondias pinnata* (Family: Anacardiaceae) and *Terminalia chebula* (Family: Combretaceae); and stem, leaves of *Hedyotis pinifolia* (Family: Rubiaceae), were purchased from local market depicted in Table 1.

### **Solvent Extraction:**

Thoroughly washed mature leaves were shade dried and then powdered with the help of a blender. 2.0 g of the powder was filled in a thimble and extracted successively with methanol using a Soxhlet extractor for 48 hr. All the extracts were concentrated using a rotary flash evaporator and preserved at 5° C in airtight bottle until further use.

### **Growth and Maintenance of Test Microorganism for Antimicrobial Studies:**

Bacterial cultures were obtained from the American Type Culture Collection (ATCC). These included *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 19433), *Listeria monocytogenes* (ATCC 19118), *Listeria innocua* (ATCC 33090), *Bacillus subtilis* (ATCC 6633), *Micrococcus luteus* (ATCC 10240), *Enterobacter aerogenes* (ATCC 13048) and *Enterococcus cloacae* (ATCC 35030). The bacteria were activated and cloned three successive times in nutrient agar and stored on nutrient agar slants at 4° C. Subsequent cultivation and tests were done on nutrient agar medium.

### **Preparation of the tested bacteria:**

The test microorganisms were pre-cultured in nutrient broth overnight in a rotary shaker at 37° C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A<sub>610</sub> nm) to about 10<sup>6</sup> colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used.

### **Antibacterial activity:**

Methanol extracts of *Ocimum gratissimum* (Family: Lamiaceae), *Terminalia chebula* (Family: Combretaceae), *Spondias pinnata* (Family: Anacardiaceae) and *Heydotis pinifolia* (Family: Rubiaceae) were tested by agar well diffusion method. Different concentrations of each of the extracts were prepared reconstituting with methanol. The constituted extract was diluted to give 100 mg/ml, 75 mg/ml, 50 mg/ml, 25 mg/ml, and 6.25 mg/ml. 100 µl (10<sup>6</sup>cells/ml) inoculum of each of the tested microorganisms was seeded into nutrient agar medium by spread plate method. After solidification, five wells (5 mm diameter) were punched into the agar plate by using a 6 mm sterile cork borer. 100 µl of each of the extracts was separately added into the wells using a sterile syringe and allowed to diffuse at room temperature. Streptomycin sulphate (10 µg ml<sup>-1</sup>) and methanol solvent (100 µg ml<sup>-1</sup>) were used as positive and negative control respectively. The plates were incubated at 37° C + 2° C for 24 hours for bacterial activity. The diameters of the inhibition zones were measured in mm. The MC (Minimum Concentration) was regarded as the lowest concentration of the extract which didn't permit the growth of susceptible bacteria (Vila, 2002).

### **RESULTS:**

Results obtained in the present study relieved that the tested four medicinal plants extracts possess potent antibacterial activity against the nine bacterial strains investigated viz. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Micrococcus luteus*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Listeria innocua* and *Listeria monocytogenes*. The bioactivity measured in terms of zone of inhibition exhibited by the different extracts against the respective bacterial strains is presented in table-1.

The methanol extract of *Ocimum gratissimum* L. showed significant activity against *B. subtilis* (21mm of clear zone) and least activity recorded in *E. cloacae* measured 14 mm. In case of *Terminalia chebula*, highest activity was noticed against *B. subtilis* (23mm of clear zone) followed by *L. innocua* (16mm) and least in *E. faecalis* (12mm). *Spondias pinnata* extract was found to be more effective against *P. aeruginosa* (17mm). *Heydotis pinifolia* extracts showed almost similar zone of inhibition against all the tested bacteria except *B. subtilis* which showed highest activity (22 mm). (FIG 1)

### **DISCUSSION:**

From the above results it can be concluded that the different plant extract showed varying degree of antibacterial activity against Gram<sup>+</sup> positive and Gram<sup>-</sup> negative bacteria. Thus these plants have great potential as antimicrobial agents and can be used in the treatment of infectious diseases caused by resistant microorganisms. Such screening and identification of various natural organic compounds present would possibly lead to the successful prediction of the active agent present and could further lead to drug

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development. This study is a preliminary evaluation of antimicrobial activity of the plants and further phytochemical studies are required to establish the types of compounds responsible for the antimicrobial effects of these medicinal plants.

In order to identify the plants for their antibacterial properties, four medicinal plant species namely, *Ocimum gratissimum*, *Terminalia chebula*, *Spondias pinnata* and *Heydotis pinifolia* were tested against nine different pathogenic strains using the agar well diffusion method. These plant extracts showed varied inhibition zones against all the strains tested. Five dilutions (6.25%, 25%, 50%, 75%, 100%) were used in the assay. Antibacterial activity was found varying with the bioactive compounds of different species and different pathogenic bacteria.

In this report, it has been observed that Gram- positive bacteria were found to be more susceptible as compared to the Gram-negative bacteria. This findings were in agreement with observations by Cos et al.,2006 that Gram-negative bacteria are much more resistant than Gram-positive bacteria. It has been stated that the possible mechanism behind the sensitivity of Gram-positive bacteria may lie in the significant differences in the outer layers of Gram-negative and Gram-positive bacteria. Gram-negative bacteria possess an outer membrane and a unique periplasmic space (Duffy and Power, 2001) whereas the Gram-positive bacteria found to be much more susceptible since they have only an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer and Gerhardt, 1971). The presence of some metabolic toxins or broad-spectrum antibiotic compounds might confer for its antibacterial activity. Studies reported that tannins could be one of the component responsible for antibacterial activity (Al Genaidy,1993). Some studies claim that the phenolic compounds present in spices and herbs might confer the plants with its therapeutic activities (Hara-Kudo et al., 2004). In particular, flavonoids have been reported to have antibacterial activity against both Gram-positive and Gram-negative bacteria including antibiotic resistance bacteria (Bylka et al., 2004). A high percentage of cavaicrol and thymol present in the extracts of certain aromatic plants (e.g. the mint family, Lamiaceae) reported to have a higher efficacy against bacterial strains (Rasooli et al., 2006). This in vitro study corroborated the antimicrobial activity of *Ocimum gratissimum*, *Terminalia chebula*, *Spondias pinnata* and *Heydotis pinifolia* in Ayurveda. These findings support the traditional knowledge of local users and is a preliminary scientific validation for the use of these plants for antibacterial activity.

#### **CONCLUSION:**

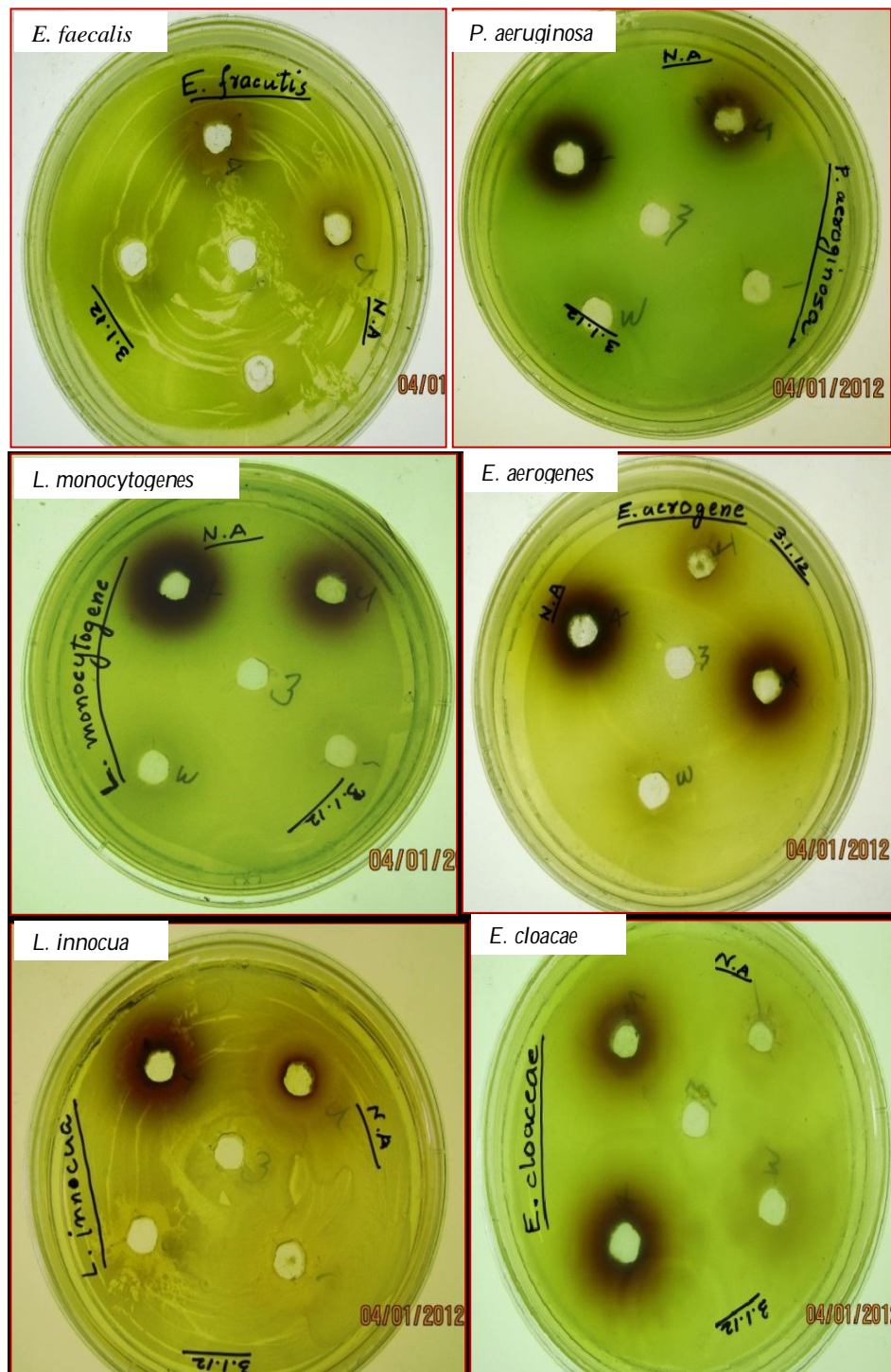
In conclusion, all of the plant extracts tested in this study showed more or less antibacterial activities. The methanol extract of all the four plants showed highest activity against *B.subtilis* among the microorganisms tested. The results of the investigation support the traditional claim of the plants. Thus further work should be performed in identifying the active ingredients present and its solubility, isolation and characterization of the active principle responsible for the activity. Thus these plants could be a possible source to obtain new and effective herbal medicines to treat infections caused by resistant strains of microorganisms. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties. Further studies are needed to isolate, characterize and elucidate the structure of the bioactive compounds present for antimicrobial drug formulation. The constituent molecules obtained from these extracts and fractions can be good candidates for structure elucidation and the structural modification for desired antibacterial molecules with desired efficacy and safety profile.

Test strain	Zone of inhibition																							
	<i>Ocimum gratissimum L.</i>						<i>Terminalia chebula</i>						<i>Spondias pinnata</i>						<i>Hedyotis pinifolia</i>					
	100 mg/ml	75 mg/ml	50 mg/ml	25 mg/ml	6.25 mg/ml	MC	100 mg/ml	75 mg/ml	50 mg/ml	25 mg/ml	6.25 mg/ml	MC	100 mg/ml	75 mg/ml	50 mg/ml	25 mg/ml	6.25 mg/ml	MC	100 mg/ml	75 mg/ml	50 mg/ml	25 mg/ml	6.25 mg/ml	MC
<b>LI</b>	16.07	14.4	10.32	9.56	7.53	6.25	17.8	15.74	12.62	8.48	6.87	6.25	12.48	9.65	7.0	8.98	-	25	14.0	12.7	9.73	8.12	-	25
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	1	0.40	0.42	0.42	0.5	0.42	1	0.5	0.85	0.5			0.55	0.72	0.45	0.48			0.89	0.59	0.73	0.56		
<b>SA</b>	15.53	12.6	10.58	7.43	6.79	6.25	14.2	12.32	9.8	6.86	5.46	6.25	13.86	11.68	10.32	7.56	6.07	6.25	13.29	10.7	9.26	9.83	-	25
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.5	0.5	1.3	0.85	1.5	0.5	1	1	0.65	0.35			0.61	0.72	0.88	0.78	0.50		0.76	0.67	1.03	0.65		
<b>PA</b>	17.58	14.7	12.0	9.45	8.72	6.25	16.7	13.86	10.24	9.47	7.07	6.25	17.03	15.75	12.55	10.3	7.78	6.25	12.66	10.4	9.24	7.5	7.06	6.25
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.45	0.64	0.62	0.45	1	0.5	0.37	0.5	0.68	0.54			0.60	0.52	0.52	0.42	0.66		0.57	0.29	0.70	0.57	0.52	
<b>EF</b>	14.41	11.7	9.61	8.81	5.05	6.25	12.5	10.76	9.37	6.50	-	25	17.5	14.67	11.94	9.7	7.21	6.25	10.11	9.04	7.78	7.21	-	25
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.54	1.0	0.45	0.42	0.58	0.78	0.44	0.36	0.44				0.45	0.62	0.80	0.84	0.76		0.60	0.48	1.21	0.56		
<b>LM</b>	17.11	15.3	12.85	8.86	8.14	6.25	15.0	13.25	10.60	8.49	6.57	6.25	15.6	13.95	10.3	8.24	-	25	15.32	13.5	10.9	9.09	-	25
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.34	0.73	1.03	0.62	0.44	0.55	0.23	0.55	0.38	0.63			0.42	1.06	0.47	0.47			0.73	0.56	0.60	0.36		
<b>BS</b>	21.58	18.4	14.51	10.2	8.66	6.25	23.4	18.46	14.5	9.78	7.11	6.25	18.43	16.7	15.26	11.9	-	25	22.25	19.6	17.0	13.4	10.1	6.25
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.33	0.52	0.18	0.33	0.50	0.44	0.50	0.76	0.73	0.68			0.61	0.50	0.69	1.04			0.72	0.39	0.43	0.56	0.63	
<b>EC</b>	13.29	12.0	8.22	7.14	5.40	6.25	13.0	10.64	8.70	7.25	5.89	6.25	14.47	13.29	11.68	9.7	7.78	6.25	9.66	9.12	7.91	6.54	-	25
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.76	0.60	0.62	1.06	0.32	1.11	0.57	0.77	0.54	0.66			0.60	0.76	0.72	0.84	0.66		0.69	0.45	1.0	0.63		
<b>EA</b>	15.54	13.5	8.91	7.82	5.16	6.25	15.5	13.38	10.24	9.80	7.43	6.25	13.43	11.57	10.36	7.19	6.57	6.25	12.24	10.7	11.5	9.12	-	25
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.60	0.55	0.60	0.94	0.72	0.45	0.59	0.67	0.59	0.80			0.63	0.63	0.57	0.63	0.75		0.56	0.59	0.44	0.56		
<b>ML</b>	16.79	13.8	10.8	6.95	5.94	6.25	12.0	10.75	7.68	7.36	-	25	11.52	9.69	7.06	8.41	-	25	14.87	13.3	11.8	7.95	-	25
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	1	0.37	0.45	0.50	0.37	0.34	0.55	0.51	0.34				0.47	0.55	0.52	0.44			1.07	0.68	0.31	0.72		

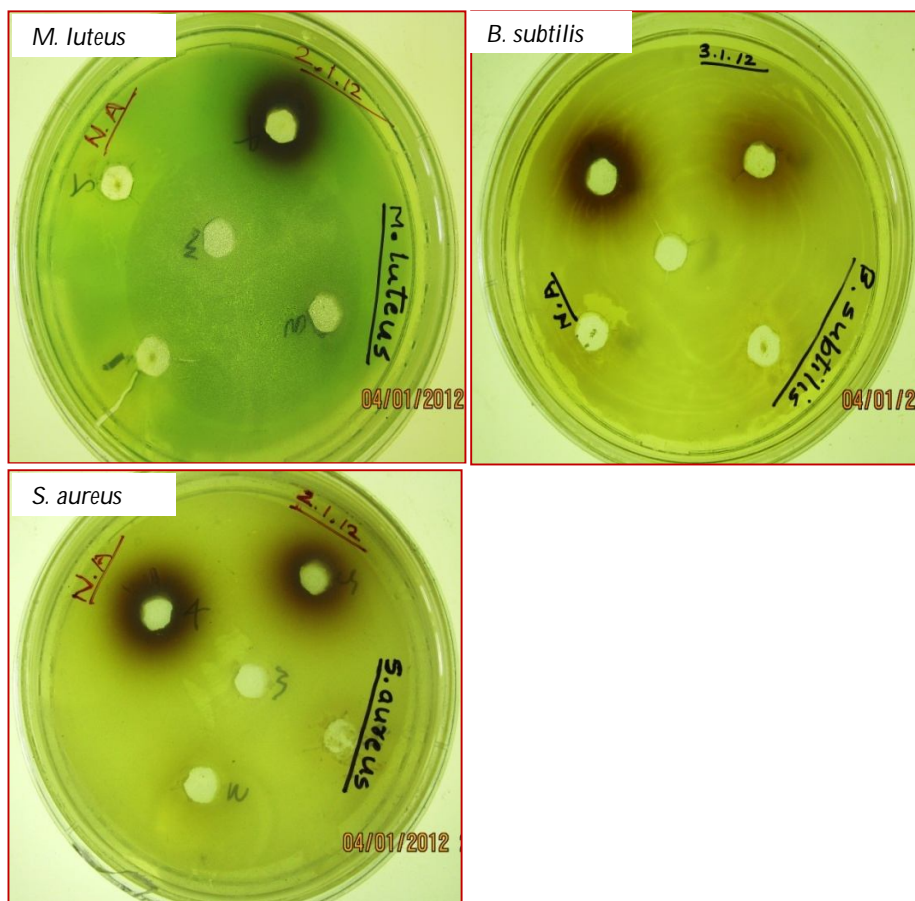
**Table 1:** Antibacterial activity of the medicinal plants against bacterial species tested by well diffusion assay.

EA- Enterococcus faecalis, SA- Staphylococcus aureus, PA- Pseudomonas aeruginosa, LI- Listeria innocua, LM- Listeria monocytogenes, BS- Bacillus subtilis, ML- Micrococcus luteus, EA- Enterobacter aerogenes, and EC- Enterococcus cloacae. MC- Minimum Concentration. Values are mean inhibition zone (mm) ± S.D of three replicate.

**FIG 1.** Preliminary screening for antibacterial activity of different plant extracts showing clear zone of inhibition.







## REFERENCE:

1. Agatemor, C. (2009). Antimicrobial activity of aqueous and ethanol extracts of nine Nigerian spices against four food borne bacteria. *Elec. J. Environ. Agric. Food Chem.* 8(3): 195-200.
2. Al-Genaigy, M. (1993). *Jordan Wild Plants and their Uses*. Amman Jordan: Al Aseel Publication 198 pp.
3. Ali, N.A.A, Julich, W.D., Kusnick, C., Lindequist, U., (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacology* 74, 113-123.
4. Bibhabasu, H., Santanu, B., Nripendranath, M. Antioxidant and free radical scavenging activity of *Spondias pinnata*. *BMC Complementary and Alternative Medicine* (2008); 8:63.
5. Bylka, W., Matlawska, I., Pilewski, N.A. (2004). Natural flavonoids as antimicrobial agents. *J. Amer. Nutr. Asso.* 7:24-31.
6. Correa, M.P.(1932). *Dicionario das plantas uteis do Brasil*. IBDF, Rio de Janeiro.
7. Cos, P., Vlietinck, A. J., Vanden, B. D. and Maes, L. (2006). Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. *Journal of Ethnopharmacology* 106, 290-302.
8. Cowan, M.M. (1999). Plants products as antimicrobial agents. *Clinical Microbiology Reviews*
9. De Pasquale, A. (1984) Pharmacognosy: the oldest modern science, *Journal of Ethnopharmacology*, 11,1-16.
10. Duffy, C. F. and Power, R. F. (2001). Antioxidant and antimicrobial properties of some Chinese plant extracts. *Int. J. Antimicrob. Agents* 17, 527-529.
11. Gayathri Gunalan, A., Saraswathyand K. Vijayalakshmi. Preliminary Phytochemical and Pharmacognostical Analysis of *Bauhinia variegata* Linn. leaves. *Research Journal of Pharmacognosy and Phytochemistry*; Volume 03, Issue 05, September- October, 2011.
12. Gordon, M.C., David, J.N., (2005). Biodiversity: A continuing source of novel drug leads, *Pure Appl Chem*, 77, 7-24.
13. Hara-Kudo, Y., Kobayashi, A., Sugita-Konishi, Y., Kondo, K. (2004). Antibacterial activity of plants used in cooking for aroma and taste. *J. Food Protect.* 67: 2820-2824.
14. Mahady, G.B. (2005). Medicinal plants for the prevention and treatment of bacterial infections. *Curr. Pharm. Des.*, 11: 2405-2427.
15. Mahesh, B. and Satish, S. (2008). Antimicrobial activity of some important medicinal plants against plant and human pathogens. *World J of Agri Sci.*4(5): 839- 843.
16. Onajobi, F.D. (1986). Smooth muscle contracting lipid soluble principles in chromatographic fractions of *Ocimum gratissimum*.

- J.Ethnopharmacol.*, 18:3-11.
17. Prakash, J., Jadeia, D. and Chanda, S. (2005). Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turky J Biol.* 29:203-210.
  18. Rasooli, I., Rezaei, M.B., Allameh, A. (2006). Ultrastructural studies on antimicrobial efficacy of thyme essential oils on *Listeria monocytogenes*. *Int. J. Infect. Dis.*, 10: 236-241.
  19. Rastogi, R.P. and Mehrotra, B.N. (2002). *Glossary of Indian Medicinal Plants*. National Institute of science communication, New Delhi, India, 20-25 PP.
  20. Rates, S.M.K., (2001). Plants as source of drugs. *Toxicon*, 39: 603-613.
  21. Scherrer, R., Gerhardt, P. (1971). Molecular sieving by the *Bacillus megaterium* cell wall and protoplast. *J. Bacteriol.* 107: 718-735.
  22. Sher, A.: Antimicrobial activity of natural products from medicinal plants. *Gomal J Med Sci.*: 7(1):72-78 (2009).
  23. Vila, J., Ruiz, J., Gallardo, F., Vargas, M., Soler, L. and Figueras, M.J. (2003). *Aeromonas* spp. and traveler's diarrhea: clinical features and antimicrobial resistance. *Emerging Infectious Diseases*, 9: 552-559.