DOSE RESPONSE CURVE OF PLANTS EXTRACTS AGAINST THE HUMAN PATHOGENS

Agha Asad Noor[1], Abdul Ghaffar Memon[2] and Sikander Khan Sherwani[3]
[1]*Department of Microbiology, University of Sindh Jamshoro(Sindh) Pakistan*
[2]*Department of Microbiology, University of Sindh Jamshoro(Sindh) Pakistan*
[3]*Department of Microbiology, Fedral Urdu University of Science & Technology, Karachi(Sindh) Pakistan*

ABSTRACT

Plants have been significantly used since the ancient times due to the presence of diverse chemical compounds having therapeutic nature against various disorders and the infectious diseases of humans. Herbs and spices have been known for human diet but spices possess some allergic chemicals that's why the search for drugs and dietary supplements derived from plants has more importance now a days. The present work shows that the antibacterial activity of methanolic garlic, ethanolic ginger and ethanolic neem extracts against the 21 human pathogens isolated from various specimens against various antibiotics. A disc impregnated in a mixture of Cefataxime (crude) was used as standard. The antibacterial activity was observed in all extracts and maximum effect of garlic on *Staphylococcus aureus* (90%), ginger on *Staphylococcus aureus* and *Klebsiella pneumoniae* (86 and 85 %) and neem on *Staphylococcus aureus* (95%) as compared to other test isolates respectively. Neem was reported as the most effective plant extract having least MIC with greater percentage of antibacterial activity as compared to the other test extracts when tested by disc diffusion and well diffusion methods. The minimum inhibitory concentration revealed 4, 6, 3, 3, 2 garlic, 6, 6, 5, 4, 3 garlic and 2, 3, 1, 2, 1 mg of neem extracts when suspended in 100 ml of the cell suspension 1×10^{-6} colony forming unit (cfu) respectively. It was concluded that Neem extract has strong and most effective antibacterial property as compared to ginger and garlic.

Key words: Medicinal plants, antimicrobial activity, MIC, well diffusion method.

INTRODUCTION

The Unani system of medicine has been flourishing in many central Asian countries including China, India, Sri Lanka, Pakistan, Afghanistan (Hamarneh, 1999). Plants have been a source of herbal remedies throughout the history of mankind. The drug development from plants could be useful in the demand for new medicines and the reduced side effects (Srivastava et al., 2000; Mukherjee an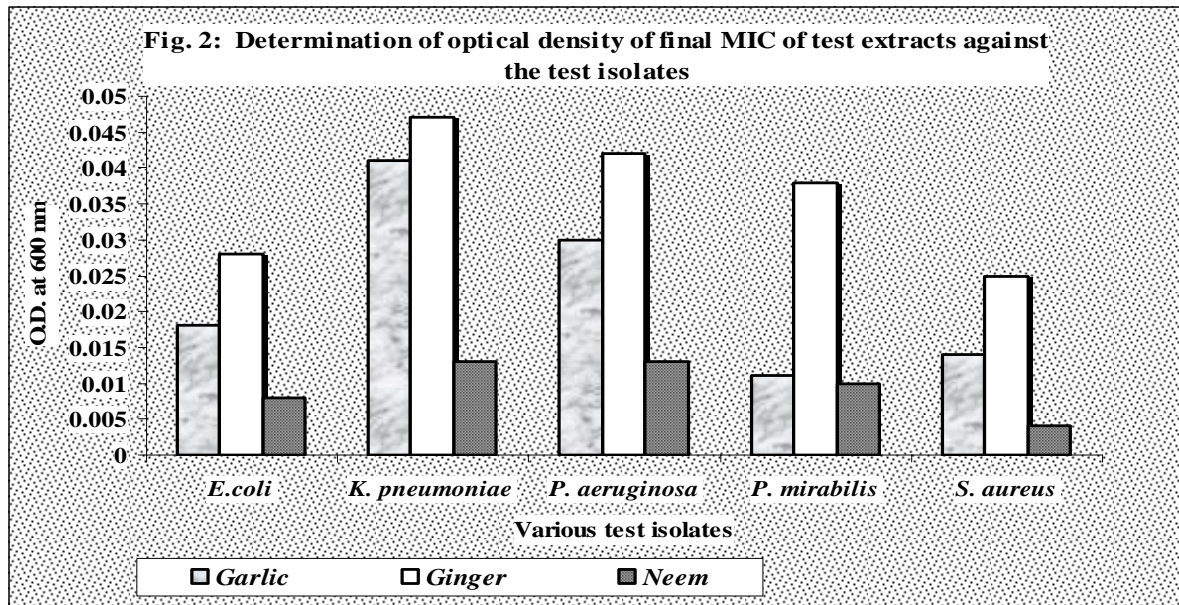d Wahile, 2006) The herbs and spices, which are known for the preservative and having medicinal properties are a significant part of the human nutrition (Draughon, 2004). Infectious diseases are the main cause of unknown mortality around the world. Many are known to be treated with herbal treatments that are the main cause (Kumar et al., 2008; Sakata et al., 2009; Wadud et al., 2007). Different workers of various disciplines including ethanopharmacologists, botanists, microbiologists and natural products chemists are developing the

photochemicals for the remedies of various infectious diseases (Jain et al., 2009). The increasing demand for food and medicine has forced the alternatives for rapid multiplication of the food and medicinal plants (Mughal, et al., 1999) for the treatment of bacterial, viral and fungal infections (Mishal and Somani, 1999). Hazni, et al., (2008), Kumar et al., (2008) and Barbosa et al., (2009) reported the continuous and urgent need to discover the antimicrobial compounds for new infectious diseases.

The essence of our work is based on three different aspects; isolation of clinical isolates from various specimens; extraction of herbal extracts that are effective against the human pathogenic bacteria of G+ve and G-ve group and to explore the right antibacterial effects of antibiotic discs and herbal extracts such as garlic, neem and ginger prepared in ethanol, methanol, acetone and distilled water by disc diffusion method, well diffusion method and spectroscopy. This work was aimed to provide awareness of the pathogenic flora from various specimens, to study the comparative effects of various allopathic medicines with the herbal extracts on the growth of clinical isolates by disc diffusion technique and spectroscopy, to explore the growth inhibitory concentration of medicinal extracts, to explore awareness of medicinal used of test plants among the common people for protection from various diseases without any side effects and to motivate pharmaceutical companies for the promotion of herbal medicines as chemotherapeutic agent.

	Garlic	**Ginger**	**Neem**
E.coli	0.018	0.028	0.008
K. pneumoniae	0.041	0.047	0.013
P. aeruginosa	0.03	0.042	0.013
P. mirabilis	0.011	0.038	0.01
S. aureus	0.014	0.025	0.004

Fig. 2: Determination of optical density of final MIC of test extracts against the test isolates

METHODOLOGY

COLLECTION OF SPECIMENS

Forty two clinical specimens of patients were collected from different hospitals of Hyderabad and Jamshoro for the isolation, identification of clinical isolates particularly g+ve and g-ve bacteria following the standards of United States Occupational Safety & Health Administration (OSHA) Laboratory Safety Guidelines (OSHA 3404-2011). The samples were (15 urine samples), collected by allowing the patients (male) to wash the tips of penis with luke warm water and to void few ml of urine in order to collect mid stream urine in narrow mouthed sterile bottle (09 pus samples) by commercially available cotton swabs (Oxoid) from the patients of skin infections, knife cut and gun shot (11 samples of nasal swab) by allowing the patients to rest their heads to expose their

nasal passage / nostrils in upward direction and slow insertion of swab to induce sneeze in order to release the pathogenic microorganisms. The swab is transported to laboratory in newly prepared synthetic enriched transport medium (SET medium) containing meat extract 1, riboflavin 0.2, biotin 0.2, asparagine 0.1, peptone 1, glucose 1 gram / liter of distilled water prepared in sterile test tubes containing 5 ml of this medium. pH of the medium was adjusted at 7.2. Seven (07) samples of sputa were also collected from the chest ward by allowing the patients to wash their mouth with luke warm water. A sterile wide mouthed bottle was opened near the mouth of the patient and allowed them to expectorate into a bottle and brought to laboratory for processing. All clinical specimens were refrigerated and processed

accordingly (Baron et al., 1994).

LABORATORY PROCEEDINGS
All the glassware were washed with distilled water using liquid detergent, rinsed with chromic acid and again washed with tap water, autoclaved, dried in hot air oven for further process. Various laboratory media and a Synthetic Enriched Medium (SET medium) were prepared by putting all the chemical ingredients in 300 ml conical flask (w/v and v/v) and autoclaved The Tryptic soy blood agar were prepared after sterilization and cooling at 45- 50°C this medium was supplemented with 5% of blood and mixed gently for 2-3 minutes. Media were kept in incubator for 24 hours at 37°C to confirm the sterility of all the preparations.

Laboratory techniques
All specimens were transported to Microbiology Research Laboratory, University of Sindh Jamshoro and were processed initially by gram staining technique as described by Wistreich and Lechtman, (1980); Cheesbrough, (2000) and Steinbach and Shetti, (2001). Regular processing of various specimens was under taken hygienically in laminar air flow system under their respective laboratory codes. Urine (Code UR 1-15) samples were inoculated on the surface of blood agar, C.L.E.D. medium and MacConkey's agar with sterile wire loop carrying approximately 0.1 ml of sample. Pus swabs* (Code PS 1-9) were applied for gram staining and then for culturing on blood agar, chocolate agar, MacConkey's agar plates. Nasal swab* (Code NS 1-11) were applied for gram staining and then for culturing on blood agar, chocolate agar, brain heart infusion agar, MacConkey's agar plates. Sputum* (Code S 1-7) samples were examined microscopically by gram staining technique and acid fast staining, later cultured on blood agar, chocolate agar, tryptic soy agar, MacConkey's agar, EMB agar plates. Two sets of plates were used one set was incubated aerobically and other for anaerobic incubation with 5-10% CO_2 for 24 hours at 37°C. Colonies were sub-cultured separately on different media for pure culture study. All plates were examined for cultural characters and again gram staining technique was applied for confirmation of previous results.

Biochemistry of clinical isolates
Biochemical investigations were done by different tests. They clinical isolates of urine specimen *(9 isolates: UR - 1, 2, 3, 7, 9, 10, 13, 14, 15)*, pus swab *(5 isolates: PS - 4, 5, 6, 8, 9)*, nasal swab *(4 isolates*: NS - 2, 5, 6, 11) and sputum *(3 isolates*: S - 1, 5, 7) were inoculated separately on triple sugar iron (TSI) slopes, Urea agar slopes, SIM medium deeps. These isolates were also inoculated for Indole, Methyl Red, Vogaus Prauskeur's and Simmon's citrate (IMViC), catalse, coagulase, oxidase tests (Wistreich and Lechtman, 1980; Washington, 1981; Case and Johnson, 1984; Khan, 1999; Cheesbrough 2000),

PREPARATION OF HERBAL EXTRACTS
The preparation of extracts was done according to Jain et al.,(2009) with slight

modifications. The garlic, ginger were purchased from local market, washed with water and cut into small visible pieces separately, which were soaked in 100 ml of luke warm water separately for 24 hours followed by boiling till the volume was reduced to one-fourth (25 ml). Neem extract was prepared according to Nostro et al., (2006) and Koona and Budida, (2011) by washing the leaves with running tap water followed by sterile distilled water. The material was chopped into small pieces and then air dried on a filter paper sheets under the shade for 20-30 days till the leaves became fully dried.

This material was powdered and allowed for preparation of ethanolic, methanolic, acetone and distilled water extracts. They were maintained in refrigerator for further processes.

ANTIMICROBIAL ACTIVITY OF HERBAL EXTRACTS
Antibiotic assay (Control)
Whattman's No.1 filter paper discs of 6 mm diameter, soaked in hot distilled water. The distilled water was removed and the discs were kept in hot air oven for 2 hours at 80°C. Commercially available antibiotic cephalosporin third generation (Cefotaxime) was used as standard / control. Antibiotic assay was performed by soaking prepared filter paper discs in cephalosporin. After 10 minutes these discs were removed and kept in laminar air flow system for 2-4 hours (Nostro et al., 2000; Baris et al., 2006; Basri and Fan, 2005). Impragnated discs were placed in the center of Mueller Hinton agar (MHA)

plates, which were separately lawned by all clinical isolates (*Proteus mirabilis, Escherachia coli, Klebsiella pneumoniae, Pseudomonas aeroginosa and Staphylococcus aureus*) and kept in incubator at 37ºC for 24 hours for the determination of zone size against cephotaxime control.

ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS
Isolates were tested on three herbal extracts garlic (*Allium sativum L.*), ginger (*Zingiber officinale L.*) and neem (*Azadirachta indica L.*), dissolved in four different solvents viz. distilled water, acetone, methanol and ethanol e.g. by Kirby-Bauer disc diffusion (Beyer, 1959, 1966) and well diffusion method (Jain et al., 2009; Das, 2010; Mehrotra et al., 2010).

Disc diffusion (DD) method
Whattman's filter paper discs were placed separately in each herbal extract (0.2 ml approx) of garlic, ginger and neem prepared in distilled water, acetone, methanol and ethanol for 5 minutes. Isolates were lawned on Mueller Hinton agar (MHA) plates separately and the impregnated discs were kept in the center of the culture plates by sterile forceps, discs were gently pressed to intact them with the agar surface. These plates were incubated at 37°C for 24 hours along with the plates of negative and positive control. Next day zones of extracts dissolved in various solvents were measured in mm and compared with the results of control

antibiotic.

Well diffusion (WD) method

The antibacterial activity of the crude extracts was also observed according to Igbinosa et al., (2009). The clinical isolates were initially inoculated in nutrient broth for 18 h and standardized to 0.5 McFarland standards (10^{-6} cfu / ml) Two hundred microliter of the standardized cell suspensions were spread on a Mueller-Hinton agar (Valgas et al., 2007) and the wells were then bored into the agar using a sterile 6 mm diameter cork borer. Approximately 100 µl of the crude extract at 10 mg / ml. allowed to stand at room temperature for about 2 h and then incubated at 37°C. Zone were observed after 24 h. incubation at 37°C and compared with positive controls of Cefataxime 1mg / ml (Igbinosa et al., 2009) and negative control DMSO 10 µg / ml (Koona and Budida, 2011) respectively.

Dose response curve (DRC)

The minimum inhibitory concentration (MIC) was determined to observe the specific dose for growth inhibition of the test extracts. The methods of Doughari and Manzara (2008), Adegoke**,** et al., (2010) were used with some modifications. Three sets of 10 conical flasks were prepared with standardized broth culture of the clinical isolates ($1x10^{-6}$ CFU/ml) containing 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 mg / 100 ml of test dried extracts. These tubes showing MIC were further observed by spectroscopy at 600 nm (Stubbings et al., 2004) by setting the spectrophotometer at 0.000 against the optical density of

standards (nutrient broth and extracts at their respective concentrations)

RESULTS

Isolation and identification of clinical isolates.

The specimens were investigated for the isolation and identification of most frequent pathogens in various clinical specimens and their antibiotic sensitivity and resistant pattern by antibiotic discs. Our observations revealed the number of isolates as *E. coli (09), Pseudomonas aeruginosa (02) , Proteus mirabilis (04); Staphylococcus aureus (04) Pseudomonas aeruginosa (02); Klebsiella pneumoniae (03); Staphylococcus aureus (01)* from 20, 10, 04 and 06 samples of urine, pus, nasal swabs and sputum respectively. The cultural characters on various media of the test isolates were identified according to the Bergey's manual of determinative bacteriology.

Antimicrobial sensitivity zones (mm) of antibiotics and test extracts.

Various antibiotics (Table-1) were used to determine sensitivity pattern (sensitive, intermediate and resistant) on all clinical isolates. Our observations revealed the sensitive (20-27), intermediate (15-19) and resistant below (15) mm diameter zones against the standard antibiotic Cefotaxime by disk diffusion method. All ethanolic and methanolic plant extracts (garlic, ginger and neem) were also tested for antimicrobial activity by disc diffusion and agar well diffusion methods (0.1 ml in

each well) showing the various percentage of their respective potency (Table-2, ig. 1).

Antimicrobial activity of extracts.

All extracts were tested by disc diffusion and well diffusion methods. The observations revealed 15.5, 15, 14.5, 13, 18.5 mm; 15, 18, 12.5, 15, 17 mm and 18, 15.5, 15, 16.5. 20 mm mean zones of methanolic garlic, ethanolic ginger and neem extracts against *E. coli, Klebsiella*

pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis and *Staphylococcus aureus* respectively. Our observations of minimum inhibitory concentration revealed 4, 6, 3, 3, 2 garlic, 6, 6, 5, 4, 3 garlic and 2, 3, 1, 2, 1 mg of neem extracts when suspended in 100 ml of the cell suspension respectively (Table-3). Optical Density (O.D. 600 nm) of the last flasks showing no growth was also determined (Fig.2).

	Garlic	**Ginger**	**Neem**
E.coli	0.018	0.028	0.008
K. pneumoniae	0.041	0.047	0.013
P. aeruginosa	0.03	0.042	0.013
P. mirabilis	0.011	0.038	0.01
S. aureus	0.014	0.025	0.004

Fig. 2: Determination of optical density of final MIC of test extracts against the test isolates
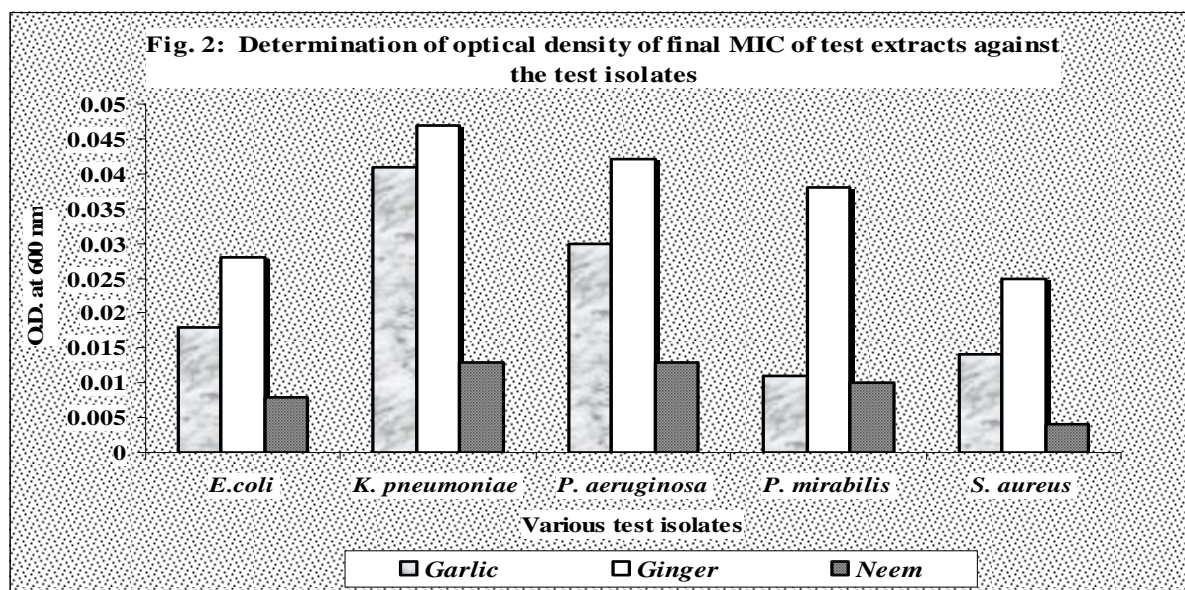
Table-1:   Determination of various zones of different antibiotic discs on clinical isolates by Kirby-Bauer (disc diffusion) method on Mueller Hinton agar showing the zone size sensitive, intermediate, and resistant.

Clinical Isolates	Sensitivity pattern of various antibiotics used on clinical isolates	
E. coli	Sensitive	Nalidixic acid, Cephradine, Chloramphenicol, Cefixime, Fosphomycin, Ofloxacin, Ciprofloxicin, Cephalothin, Nitrofurantoin, Piperacillin, Tetracycline, Trimethoprim, Ceftriaxone, Lovastatin
	Intermediate	Amikacin, Amoxicillin, Ampicillin, Gentamycin.
	Resistant	Erythromycin, Vancomycin, Tetracycline, Lincomycin, Ampicillin, Oxacillin, Penicillin-G, Azithromycin
Klebsiella pneumoniae	Sensitive	Ofloxacin, Cefixime, Cephradine, Nalidixic acid, , Cephalexin, Chloramphenicol, Tobramycin, Cephalosporin, Ciprofloxicin, Cefaclor
	Intermediate	Vancomycin, Clathromycin, Amoxicillin, Azithromycin, Fosphomycin, Ceftriaxone
	Resistant	Erythromycin, Tetracycline, Cefotaxime, Lincomycin, Ampicillin, Co-trimaxazole, Oxacillin, Gentamycin, Penicillin-G, Lovastatin.
Pseudomonas aeruginosa	Sensitive	Amikacin, Ciprofloxocin, Piperacillin, Tobramycin, Ofloxacin, Cephradine, Ciprofloxacin, Oflaxacin, Cephalosporin, Fosfomycin, Chloramphenicol, Cefaclor
	Intermediate	Cephtriaxone, Cephtriaxone, Gentamicin, Ampicillin, Norfloxocin, Cefixime, Gentamycin, Erythromycin, Clathromycin, Azithromycin, Tetracycline, Nalidixic acid, Imipenem
	Resistant	Erythromicin, Nitrofurantoin, Norfloxacin, Clindamycin, Gentamycin, Oxocillin, Vancomycin, Lovastatin, Penicillin-G, Ampicillin, Tetracyclin, Trimethoprim
Proteus mirabilis	Sensitive	Ofloxacin, Cephradine, Cefixime, Ceftriaxone, Ciprofloxicin, Ampicillin, Tobramycin, Fosfomycin, Cefaclor, Piperacillin
	Intermediate	Nalidixic acid, Chloramphenicol, Imipenem, Cephradine, Vancomycin, Nitrofurantoin, Lovastatin.
	Resistant	Clarithromycin, Erythromycin, Gentamicin, Ampicillin, Cefotaxime, Lincomycin, Amikacin, Tetracyclin, Penicillin, Penicillin-G
Staph. aereus	Sensitive	Amikacin, Ampicillin, Cefitriaxone, Minocin, Amoxycillin, Chloramphenicol, Penicillin-G, Imipenem, Erythromicin, Norfloxacin, Tetracycline,
	Intermediate	Gentamycin, Cefizox, Gentamicin, Nitrfurantoin, Septran, Amikacin, Fosfomycin, Lincomycin, Vancomycin, Oxicillin, Trimethoprim, Amoxicillin
	Resistant	Nalidixic acid, Lovastatin, Cephradine

Table-2 Determination of zone of growth inhibition of clinical isolates (*E.coli-I, Klebsiella pneumoniae-II, Pseudomonas aeruginosa-III, Proteus mirabilis-IV, Staphylococcus aureus-V),* on standard antibiotic (control) Cefotaxime by disk diffusion (DD) and well diffusion (WD) methods.

Clinical Isolates	Zone of inhibition of standard antibiotic (mm)	Zone of inhibition (mm) by plant extracts								
		Methanolic garlic			*Ethanolic ginger*			*Ethanolic Neem*		
		DD	WD	M	DD	WD	M	DD	WD	M
I	21	16	15	15.5	17	15	16	19	17	18
II	20	13	17	15	16	18	17	17	14	15.5
II	16	16	13	14.5	14	11	12.5	17	13	15
IV	17	14	12	13	13	15	14	17	16	16.5
V	21	20	17	18.5	19	17	18	21	19	20

Key. **"M"** means duplicate results

Table-3: Determination of MIC of garlic, ginger and neem extracts on (*E.coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus at various concentration (mg) when suspended in 100 ml of the broth culture.*

Clinical Isolates	Minimum Inhibitory Concentration (mg)		
	Methanolic garlic	Ethanolic ginger	Ethanolic Neem
E.coli	4	6	2
Klebsiella pneumoniae	6	6	3
Pseudomonas aeruginosa	3	5	1
Proteu mirabilis	3	4	2
Staphylococcus aureus	2	3	1

DISCUSSIONS

Plant essential oils and extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens (Joshi et al., 2011). This work showed that the plant extracts inhibited bacterial growth but their effectiveness varied. The antimicrobial activity of many plant extracts has been previously reviewed and classified as strong, medium or weak (Zaika, 1998). In this study we routinely

tested various antibiotic discs (Table-1) of different potencies on the clinical isolates to observe the sensitive, intermediate and resistant zone sizes of the used discs in order to compare the zone sizes of test herbal extracts. These antimicrobial agents belong to different groups viz. cephalosporins, aminoglycosides, macrolide etc. In-vitro antibiotic assay i.e disc diffusion (DD) and agar well diffusion (WD) methods (Kirby-Bauyer, 1966; Mehrotra et al., 2010; Adegoke et al., 2010; Koona and Budida, 2011) play a significant role to chose an specific or alternative therapy for infectious diseases. Cephalosporins contain a 7-amino cephalosporanic acid nuclus, which consists of a β-lactum ring fused to a di-hydrothiazine ring (Waxman and Strominger, 1983). The third generation cephalosporin (Cefotaxime) was used as standard (control). It is more active than first generation drugs agaist *E coli, Klebsiella and Proteus sp.* (Morel et al., 1983; Sanders et al., 1985) and *Pseudomonas aeruginosa* but less active against gram positive cocci. This is due to their potent broad spectra and stability to beta lactamases and also their ability to penetrate through the outer cell envelop of gram negative bacilli (Fass, 1983; Thornsberry, 1985). Imepenum binds to PBP-1 and PBP-2 of gram negative and gram positive causing cell elongation and lysis (Spratt et al., 1977).

Aminoglycosides and Aminocyclitols are the bactericidal, active against aerobic gram negative bacilli including *Pseudomonas sp.* (Yao and Moellering, 1991). They inhibit protein synthesis by binding to 30 S ribosomal subunit and become unavailable for translation of messenger RNA during protein synthesis, there by leading to cell death (Bryan, 1984). Quinolones including fluoroquinolones act upon DNA gyrase required for DNA replication (Hooper and Wolfson, 1989). Tetracycline inhibits protein synthesis. They enter bacteria by energy dependant process and bind reversibly to the 30s ribosomal sub-unit of the bacteria. This process blocks the access of aminoacyl-tRNA to the RNA-ribosome complex, preventing bacterial polypeptide synthesis (Cravan et al., 1969). Macrolides inhibit bacterial RNA-dependant protein synthesis by binding reversibly to the 50s ribosome subunits of susceptible microorganisms, thereby blocking the translocation reaction of polypeptide chain elongation (Oleinick and Corcoran, 1969). Our results are well acquainted with the above workers.

During this work, three extracts were prepared in water, ethanol, methanol, acetone as crude extracts. Our work further explores the antimicrobial activity of methanolic garlic, ethanolic ginger and neem extracts against the human pathogens isolated from various clinical specimens. These isolates showed sensitivity against the test extracts. This may be due to some reasons such as resistance of isolates to multiple drugs, synergetic effects of test extracts and antimicrobial compounds produced by some indigenous microflora and according to Jain et al., (2009) the difference in the potency of plant extracts may be due to

different sensitivity of food associated strains, difference in concentrations, methods of extraction and little diffusion properties or may be due to the hydrophobic nature, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. (Joshi et al., 2011).

Minimum inhibitory concentration was also determined and finally optical density of the MIC at 600 nm was also determined to make a dose response curve against the test isolates. Crude garlic *(Allium sativum L..)* extracts exhibited activity against both gram negative test isolates when the extracts were kept at room temperature for 2 hours before testing. It is reported that Ajoene, a sulfur containing compound is active against G-ve and G+ve bacteria whereas the inhibition of certain thiol-containing enzymes in the microorganisms is also reported by the action of pure allicin molecules with thiol groups. The sensitivity of these isolates to garlic also revealed that the intrinsic bio-substances in this extract are significant to various drug resistance factors of the isolates, which include beta-lactamase expression, increased pyrrolidonylarylamidase activity, aminoglycoside-modifying enzymes, and altered ribosomal binding Lipid composition of cell wall has an influence on the permeability of hydrophobic and volatile bioactive substances in garlic lead to the greater effects on bacteria. Our observation of the antibacterial activity when tested at the concentration of 4,6,3,3 and 2 mg / 100 ml of the broth culture ($1x10^{-6}$ cfu) revealed 74, 80, 84, 76, and

88 percent (%) of antibacterial effects on clinical isolates and our results of antibacterial effects of garlic are well acquainted with the observations of Rabinkov, (1998), Kathi, (2000), Cercenado et al., (1996), Paparaskevas et al., (2000), White et al., (1998) and Joshi et al., (2011).

Chrubasik et al., (2005) reported that ginger *(Zingiber officinale L.)* possess following known constituents of oleoresins (gingerols, zingibain, bisabolene), essential oil (zingiberene, zingiberole, camphene, cineole, borneol), mucilage, protein, volatile oils (bisabolene, cineole, phellandrene, citral, borneol, citronellol, geranial, linalool, limonene, zingiberol, zingiberene, camphene), Goto et al., (1990), phenolics (gingeol, zingerone), proteolytic enzyme (zingibain), vitamin B6, C, calcium, magnesium, phosphorus, potassium, linoleic acid, gum, lignin, vegeto matter, asmazone, acetic acid, sulphur (Bode et al., 2001; Chrubasik et al., 2005). Number gram-positive and gram-negative bacteria including *Proteus vulgaris, Staphylococcus aureus and Streptococcus viridans, Streptococcus pyogenes, Streptococcus pneumoniae* and *Haemophilus influenzae, Pseudomonas aeruginosa, Salmonella typhimurium* and *Escherichia coli* are sensitive to ginger due to strong antibacterial activity. *In vitro* studies have shown that active constituents of ginger inhibit multiplication of colon bacteria, which ferment undigested carbohydrates causing flatulence that may be counteracted with ginger and affects the growth of *Escherichia coli, Proteus sp,*

Staphylococci sp., Streptococci sp. and Salmonella sp. Ginger when used at the concentration 6, 6, 5, 4 and 3 mg / 100 ml of broth culture showed 76, 85, 78, 82, 86 percent (%) antibacterial effect on test isolates, which highlights the that our results are in accordance of Mascolo et al., (1989); Kikuzaki, (1994); Akoachere et al., (2002); Jagetia et al., (2003), IMCR Bulletin, (2003).

Azadirachta indica, L. (Neem) It is a natural source of insecticides, pesticides and agrochemical (Girish and Shankara, 2008) and is known as evergreen tree that has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine (Koona and Budida, 2011). More than 140 compounds have been isolated from different parts of neem (Subapriya and Nagini, 2005). The compounds have been divided into two major classes: Isopenoids and others (nonisopenoids). Isopenoids include diterpenoid and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and C-secomeliacins such as nimbin, salinin and azadirachtin. The nonisopenoids include protein and carbohydrates, sulfur compounds, polyphenolics such as flavanoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds etc (Biswas et al., 2002). Few of the compounds are known to be antibacterial in nature including Nimbidn, Nimbolite, Mahmoodin, Margolone, Margolonone, Isomagolonone (Girsh and Shankara, 2008). Our results of ethanolic extract at the MIC 2, 3, 1, 2 and 1 mg / 100 ml of

broth culture showed 86, 77, 79, 97 and 95 percent (%) antibacterial activity on test isolates *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis* and *Staphylococcus aureus* respectively. Besides gram positive bacteria, the higher antimicrobial effect of neem on gram negative bacteria may be due to the degree of depolarization and increased permeability in the lipid bilayer for the cembranoid, because G-ve bacteria have more lipid in their cell wall. The depolarization effect may be associated with hydrogen bonding on the hydroxyl group in the carboxylic functionally situated at the C-19 position in the diterpene. This antimicrobial effect may be due to presence of titerpenoids, phenolic compounds, carotenoids, steroids, valvinoids, ketones, tetra-triterpenoids azadirachtin and a spermicidal compound NIM-76. Moreover, the specific effects produced by the plant extracts against particular organism may be due to various extrinsic and intrinsic parameters (Koona and Budida, 2011; Joshi et al., 2011).

CONCLUSIONS

The present study was carried out to identify traditional plants that are effective against human pathogens viz. *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis* and *Staphylococcus aureus* and to find out the minimum inhibitory concentration of the test extracts on the growth of test isolates against the commercially available discs and cefataxime control. In the light of above observation it is concluded that the

test antibiotics have bit greater effect than the test extracts but the zone size and MIC shows a marginal difference in the effects of test extracts, which indicates that plant extracts both in purified form or formulated with other compounds can provide opportunities for launching of new antimicrobial compounds with different chemical structures and advanced mode of action for newly occurring infectious diseases. It is also concluded that the test extracts have a sufficient antibacterial activity and the given MIC. Our results of the greater effect of test extracts concluded that garlic has greater effect on *Staphylococcus aureus* (90%) whereas the ginger has greater antibacterial activity on *Staphylococcus aureus* and *Klebsiella pneumoniae* (86 and 85 %) and neem showed highest effect on *Staphylococcus aureus* (95%) as compared to other test isolates. Neem was reported as strong and most effective plant extract having least MIC with greater percentage of antibacterial activity as compared to the garlic and ginger respectively.

REFERENCES

Akoachere, J. F., Ndip, R. N., Chenwi, E. B., Ndip, L. M., Njock, T. E. & Anong, D. N. (2002). Antibacterial effect of *Zingiber officinale* and *Garcinia kola* on respiratory tract pathogens, East Afr. Med. J. 79: 588–592.

Barbosa, L. N., Rall, V. L.., Fernandes, A. A., Ushimaru, P. L., da Silva Probost, I., & Fernandes, A. Jr. (2009). Essential oils against foodborne pathogens and spoilage bacteria in minced meat. Foodborne Pathog. Dis 6: 725-728.

Baris, O., Gulluce, M., Sahin, F., Ozer, H., Kilic, H., Ozkan, H., Sokmen, M. & Ozbek, T. (2006). Biological activities of the essential oil and methanol extract of Achillea Biebersteinii Afan. (Asteraceae). Turk. J. Biol. 30: 65-73.

Basri, D. F. & Fan, S. H. (2005). The potential of aqueous and acetone extracts of galls of Quercus infectoria as antibacterial agents. Indian J. Pharmacol. 37(1): 26-29.

Baron, E. J., Peterson, L. R. & Finegold, S. M. (1994). Purpose and Philosophy. In: Bailey and Scott's Diagnostic Microbiology 9th edn. pp. 2-40.

Bauer, A. W., Perry, D. M. & Kirby, W. M. M. (1959). Single disc antibiotic sensitivity testing of Staphylococci. Arch. Int. Med. 104: 208-216.

Bauer, A. W., Kirby, W. M. M., Sherries, J. C. & Turckp, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45: 493-496.

Biswas, K., Chattopadhyay, I., Baerjee, R. K. & Bandyopadhyay, U. (2002). Biological activities and medicinal properties of neem (*Azadirachtaindica*). *Curr. Sci.* 82(11): 1336-1345.

Bode, A. M., Ma, W. Y., Surh, Y. J. &

Dong, Z. (2001). Inhibition of epidermal growth factor-induced cell transformation and activator protein 1 activation by 6-ginerol, Cancer Res. 61: 850–853.

Bryan, L. T. (1984). Mechanism of action of amino glycoside antibiotics. In: new dimensions in antimicrobial therapy edt. R. K. Root and M. A. Sande, Churchil Living Stone, Inc., NY, 17-36.

Cercenado, E., Vicente, M. F., Diaz, M. D. and Sanchez-Carrillo, C. (1996). Sanchez-Rubiales M: Characterization of clinical isolates of betalactamase-negative, highly ampicillin-resistant *Enterococcus faecialis.* Antimicrob Agents Chemother 40: 2420 - 2422.

Chang, T., Black, A, Dunky, A., Wolf, R., Sedman, A. Ladds, J. & Welling, P. G. (1988). Pharmacokinetics of intravenous and oral enoxacin in healthy volunteers. J. Antimicrob. Chemother. 21 (B): 49 – 52.

Chowdhury, A. K., Ahsan, M, Islam, S. N. & Ahmed, Z. U. (1991). Efficacy of aqueous extract of garlic and allicin in experimental shigellosis in rabbits. Ind. J. Med. Res. 93: 33–36.

Chrubasik, M. Pittler, H. & Roufogalis, B. D. (2005). Phytomedicine 12 (9):684-701.

Case, C. L. & Johnson D. R. 1984. Laboratory Experiments in Microbiology, Benjamin / Cummings Publishing Co. Inc. UK. pp. 1- 414.

Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries, vol. 2: pp. 1- 434.

Cravan, G. R., Gavin, R. & Fanning, T. (1969). Transfer RNA binding site of the 30S ribosome and the site of tetracycline inhibition. Cold spring harbor symptoms. Quant. Biol. 34: 129–137.

Das, K., Tiwari, R. K. S., & Shrivastava, D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research* 4(2): 104-111.

Draughon, F. A. (2004). Use of botanicals as biopreservatives in foods. Food Technol. Feat. 58: 20-28.

Fass, R. J. (1983). Comparative *in-vitro* activity of 3rd generation of cefelosporin. Arch. Intern. Med. 143: 1743 – 1745.

Girsh, K & Shankara, B. S. (2008). Neem-A green treasure. *Electronic Journal of Biology.* 4 (3): 102-111.

Goto, C., Kasuya, S., Koga, K., Ohtomo, H. & Kagei, N. (1990). Lethal efficacy of extract from *Zingiber officinale* (traditional Chinese medicine) or [6] -shogaol and [6]-gingerol in Anisakis larvae *in-vitro*. Parasitol. Res. 76 (8): 653-656.

Hanzi, H., Ahmad, N., Hitotsuyanagi, Y. Takeya, K., & Choo, C. Y. (2008). Phytochemical constituents fromCassia alata with inhibition against methicicillin-

resistant Staphylococcus aureus (MRSA). Plants Med. 74: 1802-1805.

Hooper. D. C., & Wolfson, J. S. (1989). Mode of action of the quinolone antimicrobial agents: a review of recent information. Rev. Infect. Dis. 11(5): 902-912.

Hamarneh, S. K. (1999). Greek: Unani-Arab-Islamic Medicine in Central Asia. A Twentieth-Century Perspective, Hamdard Medicus, Vol. XlII, No.1: 5-19.

Jagetia, G. C., Baliga, M. S., Venkatesh, P. & Ulloor, J. N. (2003). Influence of ginger rhizome (*Zingiber officinale* Rose) on survival, gluthathione and lipid peroxidation in mice after whole body exposure to gamma radiation, Radiat. Res. 160: 584–592.

Jain, P., Bansal, D. & Bhasin, P. (2009). Antibacterial activity of aquous plant extracts against Escherichia coli and Bacillus subtilis. Drug Invention Today 2 (4): 220-222.

Joshi, B., Sah, G. P., Basnet, B. B., Bhatt, M. R. Sharma, D., Subedi, K. Pandey, J. & Malla, R. (2011). Phytochemical extraction and antimicrobial properties of different medicinal plants*: Ocimum sanctum* (Tulsi),*Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem) J. Microbiol. and Antimic. 3(1):   1-7.

Igbinosa, O. O., Igbinosa, E. O. & Aiyegoro, O. A. (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn) African Journal of Pharmacy and Pharmacology 3(2): 58-62.

IMCR Bulletin, (2003). Ginger: Its role in xenobiotic metabolism ISSN 0377-4910, vol. 33 (6): 57-63.

Kathi J. K. (2000). Longwood Herbal Task Force:
http://www.mcp.edu/herbal/default.htm

Khan, A. M. & Mahmood, N. (1999). Laboratory Manual of Microbiology, 5th edn. pp. 3-144.

Kikuzaki, H., Kawasaki, Y., Nakatani, N. Structure of antioxidative compounds in ginger.   (1994).   ACS-symp-ser. Washington, D.C. Am. Chem. Soc. 547: 237-243.

Koona, S. & Budida, S. (2011). Antibacterial potential of the extracts of the leaves of *Azadiracha indica* Linn. Notulae Scientia Biologicue 3(1): 65-69.

Kumar,M. S., Kirubanandan, S., Sripriya, R. & Sehgal, P. K. (2008). Triphala promotes healing of infected full thickness dermal wound. J. Surg. Res. 144: 94-101.

Mascolo, N., Jain, R., Jain, S. C. & Capasso, F. (1989). Ethnopharmacologic investigation of ginger (*Zingiber officinale*). J. Ethnopharmacol. 27: 129-140.

Mehrotra, S., Ashwani, K. & Nandi, S. S.

P. (2010). Camparative antimicrobial activities of Neem, Amla, Aloe, Assam Tea and Clove extracts against *Vibrio cholerae, Staphylococcus aureus* and *Pseudomonas aeruginosa. J. Med. Plants Res*. 4(22): 2393-2398.

Mishal, H. & Somani, R. (1999). Herbal antibacterials. *Hamdard Medicus*, vol. XLIII, No. 4: 109-112.

Morel, C., Vergnaud, M., Langard, M. M. & Dupuy, L. (1983). Cefotetan: Comparative study *in-vitro* against 266 gram negative clinical isolates. J. Antimicrob. Chemother. 11(suppl. A): 31-36.

Mughal, M. H., Ali, G., Srivastava, P. S. and Iqbal, M. (1999). Improvement of drumstick *(Moringa pterygosperma* Gaertn.). A Unique source of food and medicine through tissue culture. Hamdard Medicus, vol. XlII, No. 1: 37-42.

Mukherjee, P. K., & Wahile, A. (2006). Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. J. Ethnopharmacol. 103: 25-35.

Nostro, A., Germano, M. P., D'Angelo, V., Marino, A. & Cannatelli, M. A. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Microbiol. 30 (1): 379-384.

Oleinick, N. L., & Corcoran, J. W. (1969). Two types of binding of erythromycin to

ribosome from antibiotic sensitive and resistant *Bacillus subtilis* 168 J. Biol. Chem. 244: 727 – 735.

OSHA-3404. (2011). Laboratory Safety Guidance. pp. 1-48.

Paparaskevas, J., Vatopoulos, A., Tassios, P. T., Avliami, A., Legakis, N. J. and Kalapothaki, V. (2000). Diversity among high-level aminoglycoside resistant enterococci. J Antimicrob Chemother 45: 277 -283.

Rabinkov, A., T. Miron, L. Konstantinovski, M. Wilchek, D. Mirelman & Weiner, L. (1998). The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. Biochim. Biophys. Acta 1379: 233-244.

Sakata, H., Toyonaga, Y., Sato, Y., Hanaki, H., Nonoyama, M., oishi, T., & Sunakawa, K. (2009). Nationwide survey of the development of drug-resistance in the piediatric field: drug sensitivity of Haemophilus influenziae in Japan. *J. Infec. Chemother*. 15: 402-409.

Sanders, C. V., Greenburg, R. N. & Marier, R. L. (1985). Cefamadol and cefoxitin. Ann. Intern. Med. 103: 70-78.

Spratt, B. G., Joban, P. V. & Zimmermamm W. (1977). Binding of thlenamycin and clavulanic acid to the penicillin binding proteins of *E coli* K-12. Antimicrobiaol agents chemotherapy 12: 406 – 409.

Steinbach, W. J. & Shetty A. K. (2001). Use of the diagnostic bacteriology laboratory: A practical review for the clinician. Postgrad. Med. J. 77: 148- 156.

Srivastava, A. & Shukla Kumar Y. N. (2000). Recent development in plants derived antimicrobial constituents. A Review. J. med. Arom. Pl. Sci 20: 717- 772.

Stubbings, W. J., Bostock, J. M., Ingham, E. & Chopra, I. (2004). Assessment of a microplate method for determining the post-antibiotic effect in *Staphylococcus aureus* and *Escherichia coli* Journal of Antimicrobial Chemotherapy 54: 139–143.

Subapriya, R. & Nagini, S. (2005). Medicinal properties of neem leaves: A review. Curr. Med. Chem. Anticancer agents 5 (2): 149-56.

Thornsberry, C. (1985). Review of *in-vitro* 3rd generation of cefalosporins and other newer β-lactum antibiotics against clinically important bacteria. *Am. J. Med.* 79 (2A): 14-20.

Valgas, C., Machado de Souza, S., Smânia, E. F. A. & Smânia, A. (2007). Screening methods to determine antibacterial activity of natural products. Brazilian Journal of Microbiology 38:369-380.

Wadud, A., Prasad, P.V., Rao M. M. & Narayana, A. (2007). Evaluation of drug: A historical prospective. Bull Indian Institute. Hist. Med. Hyderabad 37: 69-80.

Waxman, D. J. & Strominger, J. L. (1983). Penicillin binding proteins and the mechanism of action of β-lactum antibiotics. Annu. Rev. biochem. 52: 825-869.

Washington, J. A. (1981). Laboratory procedures in clinical microbiology, 2nd edn., Springer-Verlag, New York, NY, pp. 113-145.

White, T. C., Marr, K. A. & Bowden, R. A. (1998). Clinical, cellular and molecular factors that contribute to antifungal drug resistance. Clin Microbiol. Rev. 11:382 - 402.

Wistreich, G. A. & Lechtman, A. D. (1980). Laboratory Exercises in Microbiology, 4th edn. Mac Millan Pub. Co., Inc. NY, pp. 1-420.

Yao, J. D. C. & Moellering, R. C. Jr. (1991). Antibacterial agents. In: Manual of clinical microbiology, 5th edn., Hausler, W. J. JR., Herrmann, K. L. Isenberg, H. D. and Shadomy, H. J. ed. Am. Soc. Microbiol. USA, pp. 1065-1098.

Zaika, L. L. (1988). Spices and herbs: their antibacterial activity and its determination. J. Food Safety. 23: 97-118.