International Research Journal of Natural and Applied Sciences
Volume-2, Issue-5 (May 2015) IF- 2.818 ISSN: (2349-4077)

COMPARATIVE STUDY OF THE EFFICACY OF LIME (*Citrus aurantifolia*), HONEY AND THEIR SYNERGY ON SOME PATHOGENS

***Akinnibosun, F. I. And Rehoboth, S. U.**

Department of Microbiology, Faculty of Life Sciences, University of Benin, P.M.B 1154, Benin City, Edo state, Nigeria.

ABSTRACT

In this study, which was set out to compare the antibacterial activity of the single effects of honey and lime (Citrus aurantifolia) with their synergy, an agar well diffusion assay was used to demonstrate the antimicrobial effects against the following pathogens; Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Escherichia coli and Bacillus subtilis. Overall analysis showed that lime juice had greater antibacterial activity against the isolates tested than honey. The MIC assay showed the values of honey to be between 50% - 75% and that of lime juice to be between 25% - 75%. The MBC of lime ranged from 50% to 100% while for honey is between 75% and 100%. The MIC and MBC values of the honey and lime juice synergistic sample, was recorded to be 25% -75% and 50% - 100% respectively. Zones of inhibition of the synergistic sample were also found to be higher than the individual samples of the honey and lime juice. It is therefore suggested that the synergistic mixture of lime juice and honey be recommended as a better option than their separate use in the treatment of infections caused by the test bacteria.

KEY WORDS: Honey, Efficacy, Lime, Synergy, Antimicrobial property.

INTRODUCTION

Diseases are the major cause of death in developing countries and accounts for 50% of it. Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, as resistant pathogens develop and spread, the effectiveness of these antibiotics are diminished. This type of bacterial resistance to these antimicrobial agents pose a very serious threat to public health, and for all kinds of antibiotics, including the major last-resort drugs, the frequencies of resistance are increasing worldwide (Levy *et al*., 2004; Mandal *et al*., 2009). Bacterial resistance to antibiotics increases mortality likelihood of hospitalization and length of stay in the hospital (Winstanley, 1997).

In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Piddock and Wise, 1989; Singh *et al*., 1992; Mulligen *et al*., 1993; Davis, 1994; Robin *et al*., 1998). However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. Therefore, alternative antimicrobial strategies are urgently needed, and thus this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants (Mandal *et al*., 2010; Basualdo *et al*., 2007). Being new, such compounds may not have the problem of microbial resistance.

Plant based antimicrobials represent a vast untapped source. The use of plant extract for medicinal treatment has become popular especially now when people are beginning to realize that the effective life span of antibiotics is limited and over prescription and misuse of traditional antibiotics are causing microbial resistance (Alam *et al.*, 2009). Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Harborne and Baxter, 1995). In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Grosvenor *et al.*, 1995; Ratnakar and Murthy, 1995; David, 1997; Saxena, 1997; Nimri *et al.*, 1999; Saxena and Sharma, 1999). Presently, nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and their extracts dominate in homeopathic or ayurvedic medicines (Murugesan *et al.*, 2011; Jabeen *et al*., 2007; Banso, 2009; Ahameethunisa and Hopper, 2010).

Lime (*Citrus aurantifolia)* is a small fruit from the Citrus family; it comes either sour or sweet naturally. Sour limes possess a greater sugar and citric acid content than lemons and feature an acidic and tart taste (Bina *et al*., 2010). The nutritional profile includes information on a full array of nutrients including carbohydrates, sugar, soluble and insoluble fiber, sodium, vitamins, minerals, fatty acids, amino acids and more. Limes contain unique flavonoid compounds that have antioxidant and anti-cancer properties. While these flavonoids have been shown to stop cell division in many cancer cell lines, they are perhaps most interesting for their antibiotic effects (Tomotake, 2006).

Honey, whose medicinal uses dates from ancient times, has lately been rediscovered as a therapy for wounds (Vishnu *et al*., 2012). Many publications attest to honey's antimicrobial properties (Akinnibosun and Itedjere, 2013). Strong solutions of honey or sugar and sugar pastes, inhibit microbial growth because of their high osmolarity but when used as dressings, they become diluted to the point where this action ceases especially in the case of *Staphylococcus aureus* (Cooper *et al*., 1999). Recently, the potent activity of honey against antibiotic resistant bacteria has further increased the interest for the application of honey, but incomplete knowledge of the antibacterial activity is a major obstacle for clinical applicability (Cooper *et al*., 2002a; Cooper *et al*., 2002b; Levy and Marshall, 2004; Walsh, 2003).

Traditional preparation of medicinal plants with antimicrobial activities have been extensively used in the West African regions (Adesuyi *et al*., 2012; Dunford *et al*., 2000; Mboto *et al*., 2009; Mythilypriya *et al*., 2007). Combined therapy is traditionally used to increase antimicrobial activity and reduce toxic effects of agents (Tallarida, 2001). This study was carried out to assess and compare the antimicrobial efficacy of the synergy of honey and lime and their use separately on some pathogenic bacteria.

MATERIALS AND METHODS

Sample collection

Lime fruits, free from decay and molds were purchased from New Benin market, and washed properly with distilled water before extracting the juice using a juice extractor. The juice was placed in sterile screw-cap bottles. Honey was purchased from a beekeeper who does bee farming in Ekosodin village, Benin City, Edo state, Nigeria. Honey samples were stored at room temperature in the dark prior to testing to prevent photodegradation.

Test organism

Bacterial cultures of the test organisms, *Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Escherichia coli* and *Bacillus subtilis* were obtained from the Department of Medical Microbiology, University of Benin teaching hospital, Benin City, Nigeria. Their identity was confirmed using cultural, morphological and biochemical test as previously described (Akinnibosun *et al*., 2009; Cheesebrough *et al*., 2002). They were maintained on nutrient agar slants at 4◦C. These test bacteria have been previously described (Prescott *et al.*, 2005; Akinnibosun *et al.*, 2008a, b).

Sample preparation:

Different concentrations: 100%, 75%, 50% and 25% of both samples were prepared after dilutions. Then equal volumes of 100% solution of lime extract and 100% honey were mixed and used for assay.

Determination of Antibacterial Activity

The crude extracts were screened for antibacterial activity i.e. determination of zone of inhibition against tested organism by agar well diffusion method. Sterile Mueller-Hinton Agar plates were inoculated with prepared inoculum with sterile cotton swab. Then with the help of sterile cork borer no. 6, wells were made in the inoculated media plate. 50 μl of the working solution/ suspension of different concentration were transferred into the well with the help of micropipette. The control was also placed in the separate well at the same time. After proper incubation, the plates were viewed for the zone of inhibition, which is suggested by clear areas without growth around the well.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The crude extracts which showed antibacterial activity, using the broth dilution method were subjected to two-fold serial dilution method to determine Minimum Inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC). For each bacterium, a set of 12 screw capped test tube containing 1ml nutrient broth was required. The test tubes were labelled as positive growth control, negative growth control and numbers 1 to 10. Different dilutions of crude extracts as 100%, 75%, 50% and 25% were made. Then two fold serial dilution of the extract was prepared each containing equal volume but decreasing concentration. 50μl of culture inoculum of test bacteria was added to each tube with the help of micropipette except negative control. All the tubes were incubated at 37 ^{0}C for 24 hours and observed for turbidity by comparing with positive and negative control. The results were interpreted on the basis of the fact that growth occurs in the positive control and any other tube in which the concentration of the extract is not sufficient to inhibit growth and lowest concentration of the agent that inhibits growth of the organism as detected by lack of visible turbidity as designated the minimum inhibitory concentration (MIC). The tubes were sub cultured on nutrient agar plate with proper label followed by incubation at 37°C for 24 hours. Then they were examined for growth of bacteria. The tube with minimum concentration of extract in which the growth was completely stopped was also clearly notified. This determined the Minimum bactericidal concentration.

RESULTS AND DISCUSSION

The present study shows the evaluation of the antibacterial properties and synergistic effect of lime and honey on some bacteria. The antibacterial activity in the study was expressed as a measure of the diameter of the inhibition growth in millimetres.

Table 1, 2 and 3 show the antibacterial activity (as shown by zones of inhibition of the different concentrations) of lime, honey and their synergy respectively. Tables 4 and 5 show the MIC and MBC of the samples on the test organisms. The results of this work revealed that the lime, honey and their synergy had antibacterial property by preventing the growth of the test organism.

Table 1: Mean Zone of Inhibition of lime (diameter in mm, including well (8.2 mm))

	Concentration (%)			
Organisms	100%	75%	50%	25%
S. aureus	30±1.7	26±0.6	18±10.6	10±0.6
S. pneumoniae	34±1.5	29±1.7	22±2.1	14±2.5
K. pneumoniae	24±1.0	19±1.5	0	0
E. coli	28±5.8	23±4.2	17±2.9	0
B. subtilis	23±2.7	17±1.0	11±2.7	0

Table 2: Mean Zone of Inhibition of honey (diameter in mm, including well (8.2 mm))

	Concentration (%)			
Organisms	100%	75%	50%	25%
S. aureus	24±1.5	19±2.1	13±1.0	0
S. pneumoniae	23±1.2	17±1.7	0	0
K. pneumoniae	17±1.7	18±2.0	0	0
E. coli	22±1.0	18±0.6	0	0
B. subtilis	20±1.7	13±1.7	0	0

Table 3: Mean Zones of Inhibition (diameter in mm, including well (8.2 mm)) in comparison with synergy, negative control (water) and positive control (Ciprofloxacin)

Isolate	100% lime	100% honey	Synergy	-ve control (water)	+ve control (CIP)
S. aureus	30±1.7	24±1.5	35±1.7	0±0.0	27±1.1
S.pneumoniae	34±1.5	23±1.2	39±1.5	0±0.0	26±1.3
K.pneumoniae	24±1.0	17±1.7	29±1.0	0±0.0	20±1.0
E. coli	28±5.8	22±1.0	33±5.8	0±0.0	25±0.6
B. subtilis	23±2.7	20±1.7	28±2.7	0±0.0	20±1.7

CIP – Ciprofloxacin.

+ve = Positive

-ve = Negative

Table 4: Minimum Inhibitory Concentrations (MIC) %

Organisms	Lime	Honey	Synergy
S. aureus	25	50	25
S. pneumoniae	25	75	25
K. pneumoniae	75	75	75
E. coli	50	75	50
B. subtilis	50	75	50

Table 5: Minimum Bactericidal Concentrations (MBC) %

Organisms	Lime	Honey	Synergy
S. aureus	50	75	50
S. pneumonia	50	100	50
K. pneumoniae	100	100	100
E. coli	75	100	75
B. subtilis	75	100	75

The antibacterial action occurred at varying concentrations on both Gram-positive and Gram-negative test bacteria (tables 1, 2 and 3), indicating that the samples had broad antibacterial spectrum (Bankole, 1992). Presence of an inhibine factor in honey which is hydrogen peroxide and flavonoids have been observed to be responsible for the antimicrobial property. The data obtained showed that the inhibitory effects of the samples on the various investigated microorganisms was dose-dependent. This observation is in agreement with the findings of Agbaje *et al*. (2006) and Akinnibosun and Itedjere. (2013), who reported that the efficacy of honey and most plant extract was concentration-dependent.

At concentration of 100% and 75% both samples had antibacterial effects on all the tested bacteria, while at 50%, lime juice had detectable antibacterial activity against all isolates except *K. pneumoniae* and honey had no detectable activity against all tested bacteria except *S. aureus*. at concentration 25%, the antibacterial effect of lime juice was only detectable against *S. aureus* and *S. pneumoniae* whole the effect of honey was not detectable in any bacterial isolates. This observation was similar to those obtained by Laid *et al.* (2013) and Bina *et al.* (2010) where decrease in zones of inhibition and reduction in efficacy was noted as a result of reduction in concentration. This result was also in agreement with those of Agbaje *et al*. (2006), who noted non-susceptibility of some test organisms to honey, due probably to emergence of resistant strains or the effect of factors such as botanical or entomological origin on the antibacterial activity of honey. It has been reported that honey from different phytogeographic regions varied in their ability to inhibit the growth of bacteria, suggesting that botanical origin plays important role in influencing the antimicrobial property.

Various researchers have shown that honey exerts antimicrobial activities against various microorganisms (Allen *et al*., 1991; Anand and Shanmugan, 1998; Cooper and Molan, 1999). Honey inhibits bacterial growth due to a number of different mechanisms; high sugar concentration, low pH, hydrogen peroxide generation, proteineceous compounds or other unidentified compounds present in the honey (Mundo *et al*., 2004). Shrinkage disruption of the bacteria may be due to its osmotic effect, low pH and also due to the presence of antibacterial substances such as inhibine (Vishnu *et al*., 2012). Lime achieves its bacterial inhibition because the lime juice contains a group of flavonoid, phytochemicals which is flavonol glycosides. Investigation of the antimicrobial activity of lime juice in combination with other herbs and alone has been studied (Mata *et al*., 1994; Castillo *et al*., 2000; Lin *et al*., 2002) and lime extract has high antimicrobial activity which has been identified (Bina *et al*., 2010). The current study further proves its antibacterial activity. Susceptibility testing with standard antibiotic, ciprofloxacin, showed zones of inhibition which ranged from 20±1.0mm to 27±1.1mm. From this observation, the samples and their synergy compared favourably with the standard antibiotic agreeing with the report of Alandejani *et al.* (2009), the MIC and MBC of the samples (tables 4 and 5) against the test organisms were also determined. The MIC varied between 25% and 75% for lime and 50% to

75% for honey while their synergy had MIC of 25% to 75%. The MBC ranged from 50% to 100% for lime, 75% to 100% for honey and 50% to 100% for their synergy. The results revealed that the synergistic use of the samples was more potent against the test organisms especially at high concentrations, considering their zones of inhibitions. The broad spectrum of activity displayed by the samples in this study appears to justify and explain the scientific basis for their uses in traditional medicine. It is hoped that this study would lead to the preparation of antibacterial drugs of natural origin for the treatment of infections caused by the test organisms.

CONCLUSION

Many currently available antifungal and antibacterial agents have undesirable toxicity, and the widespread use of these drugs has led to the rapid development of drug-resistant strains, which are the leading cause of failure in clinical applications. Natural products are both a fundamental source of new chemical diversity and an integral component of today's pharmaceutical compendium. It may be suggested that by combining both lime juice and honey, a synergistic effect would occur between both substances where one facilitates the action of the second and they both work together in disrupting cell membrane and act upon cell constituents. The present investigation provides support to the effectiveness of combined therapy between natural products. Especially in the light of the current trend in finding alternative remedies against increasing numbers of pathogenic bacteria that are resistant to current antibiotics.

ACKNOWLEDGEMENTS

The authors wish to express their sincere gratitude to the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, for the provision of materials for this work.

REFERENCES

Adesuyi, A. O., Elumm, I. K., Adaramola, F. B. and Nwokocha, A. G. M. (2012). Nutritional and Phytochemical screening of *Garcinia kola*. *Adv. J. Food Sci. Technol.* **4**(1):9-14.

Agbaje, E. O., Ogunsanya, T. and Aiwerioba, O. I. R .(2006). Conventional use of honey as Antibacterial agent. *Ann. Afr. Med.* **5**(2):78-81.

Alandejani, T. (2009). Effectiveness of honey on *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms. *Otolaryngol. Head Neck. Surv*. **141** (1): 114-118.

Ahameethunisa, A.R. and Hooper, W. (2010). Antibacterial activity of *Artemisia nilagirica* leaf extracts against clinical and phytopathogenic bacteria, *BMC Compl. and Alternative Med* **10**: 1-6

Akinnibosun, F.I. and Itedjere, E. (2013). Evaluation of the antibacterial properties and synergistic effect of Garcinia kola Heckel (Family: Guttiferae) seed extract and honey on some bacteria. *Afr. J. Microbiol. Res*. **7**(3):174-180

Akinnibosun, H. A., Akinnibosun, F. I. and Adieme, B. C. (2009). Biotheraupetic potential of aqueous and ethanolic extracts of *Solenostemon monostachyus* (P. Beauv) Brig. Leaves on some vegetative Gram-negative bacteria. *Biol. Environ. Sci. J. Trop*. **6**(3):33-37.

Akinnibosun, F. I., Akinnibosun, H. A., Ibeh, I. N. And Osaghae, F. (2008a). Antibacterial activity of *Phyllantus amarus* Schum and thorn. On five vegetative organisms. *Plant archives* **8**(2)563-568.

Akinnibosun, H. A., Akinnibosun, F. I. and German B. E. (2008b). Antibacterial activity of aqueous and ethanolic leaf extracts of *Peperomia pellucid* (L) H. B and K. (Piperaceae) on three Gram-negative bacterial isolates. *Sci. World J.* **3**(4): 33-36.

Alam, M.T., Karim, M.M. and Khan, S.N. (2009). Antibacterial activity of different organic extracts of *Achyranthes aspera* and *Cassia alata*. *J. Sci. Res.* **1**: 393-398

Allen, K. L., Molan, P. C and Reid, G. M. (1991). A survey of the antibacterial activity of some New Zealand honeys. *J. Pharm. Pharmacol*. **43**:817-822.

Anand, S. and Shanmugan, J. (1998). Antistaphylococci properties of natural honey. *Biomed.* **18:**15-18.

Bankole, A.A. (1992). The antibacterial activity of the crude seed extracts of three plants used in Nigerian ethnomedicine. University of Benin, Benin City, 45pp.

Banso, A. (2009). Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. *J. Med. Plants Res.* **3**: 82-85.

Basualdo, C., Sgroy, V., Finola, M.S. and Juam, M. (2007). Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Vet. Microbiol.* 124: 375-381

Bina, L. J., Tista, P., Anjana, S. and Kayo, D. Y. (2010). Study of antimicrobial activity of lime juice against *Vibrio cholera. Scientific World* **8:** 44-48.

Castillo, M.C., Allori, G.G., Gutierrez, R. C., Saan, O.A., Fernande, N. P., Ruiz, C. S., Holgado, A. P.and Nader, O. M. (2000). Bactericidal activity of lemon juice and lemon derivatives against *Vibrio cholerae. Biol. and Pharm Bulletin*, (10):1235-8.

Cheesebrough, M. (2002). Biochemical tests to identify bacteria in Laboratory practise in Tropical countries. Cambridge Edition pp. 63-70.

Cooper, R. A., Halas, E. and Molan, P. C. (2002b). The efficacy of honey in inhibiting strains of *Pseudomonas aeruginosa* from infected burns. *J. Burn Care Rehabil*. **23**:366-370.

Cooper, R. A. and Molan, P. C. (1999). The use of honey as an antiseptic in managing *Pseudomonas* infection. *J. Wound Care* **8**:161-164.

Cooper, R. A., Molan, P. C. and Harding, K. G. (1999). Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. *J. R. Soc. Med*. **92:**282-285.

Cooper, R. A., Molan, P. C. and Harding, K. G. (2002a). The sensitivity to honey of Gram positive cocci of clinical significance isolated from wounds. *J. Appl. Microbiol*. **93**:857-863.

David, M. (1997). Antimicrobial activity of garlic. *Antimicrob. Agents and Chem.***41:** 2286

Davis, J. (1994). Inactivation of antibiotic and the dissemination of resistance genes. *Sci*. **264**: 375-382.

Dunford, C. R., Cooper, P. and Molan, P. (2000). Using honey as a dressing for infected skin lesions. *Nurs. Times* **96**(14 Suppl):7-9.

Grosvenor, P.W., Supriono, A. and Gray, D.O. (1995). Medicinal plants from Riau Province, Sumatra, Indonesia. Part 2, Antibacterial and antifungal activity. *J. Ethnopharm* **45**: 97–111.

Harborne, S.B. and Baxter, H. (1995). *Phytochemical Dictionary*. A Handbook of Bioactive Compounds from Plants. Taylor and Francis, London.

Jabeen, R., Ashraf, M. and Ahmad, I. (2007). Evaluating the effects of cold water diffusates against *Xanthomonas oryzae* Pv. Oryzae causing bacterial leaf blight in rice. *Arch. Phytopath. and Plant Protection* **40**: 1-9.

Laïd, B. Hasan A. A., Fatiha, A., Balkees, B. and Si, M. H. (2013). Synergistic Effect of Monofloral Honeys and Essential Oils against *Pseudomonas aeruginosa. British Microbiol. Res. J.* **3**(4): 564-573.

Levy, S. B. and Marshall, B. (2004). Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med*. **10**:S122-S129.

Lin, J., Puckree, T.and Mvelase, T. P. (2002). Anti-diarrhoeal evolution of some medicinal plants used by Zulu traditional healers. *J. Ethnopharmacol* **79**:53-56.

Mata, L., Vargas, C., Saborio, D. and Vices, M .(1994). Extinction of Vibrio cholerae in acidic substrata: Contaminated cabbage and Lettuce treated with lime juice. Session de Infection-Nutrition. Universidad de Costa Rica, San Pedro. *Revista biological Tropical* **42**(3):487-92.

Mandal, S., Pal, N.K., Chowdhury, I.H. and Deb Mandal, M. (2009). Antibacterial activity of ciprofloxacin and trimethoprim, alone and in combination, against *Vibrio cholerae* O1 biotype El Tor serotype Ogawa isolates. *Pol. J. Microbiol.* **58**: 57-60.

Mboto, C. I., Eja, M. E., Adegoke, A.A., Iwatt, G. D., Asikong, B. E., Takon, I., Udo, S. M. and Akeh, M. (2009). Phytochemical properties and antimicrobial activities of combined effects of extracts of the leaves of *Garcinia kola, Vemonia amygdalina* and honey on some medically important microrganisms. *Afr. J. Microbiol. Res***. 3**(9):557-559.

Mundo, M.A., Padilla-Zakour, O. I. and Worobo, W. (2004). Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. *Int. J. Food Microbiol.* 97:1-8.

Mythilypriya, R., Shanthi, P. and Sachdanandam, P. (2007). Oral acute and subacute toxicity studies with Kalpaamruthaa, a modified indigenous preparation on rats. *J. Health Sci*. **53**(4)351-358.

Mulligen, M.E., Murry-Leisure, K.A., Ribner, B.S., Standiford, H.C., John, J.F., Karvick, J.A., Kauffman, C.A. and Yu, V.L. (1993). Methicillin resistant *Staphylococcus aureus*. *American J. Med.* **94**: 313–328.

Murugesan, S., Pannerselvam, A., and Tangavelou, A.C. (2011). Phytochemical screening and antimicrobial activity of the leaves of *Memecylon umbellatum* burm. F. *J. App. Pharm. Sci.* **1**: 42-45

Nimri, L.F., Meqdam, M.M. and Alkofahi, A. (1999). Antibacterial activity of Jordanian medicinal plants. *Pharm .Biol.***37** (3): 196–201.

Piddock, K.J.V. and Wise, R. (1989) Mechanisms of resistance to quinolones and clinical perspective. *J. Anti. Chem.* **23**: 475–483.

Prescott, L. M., Harley, J. P. and Klein, O. A. (2005). *Microbiol. 6th Edition*. McGraw Hills, New York.

Ratnakar, P. and Murthy, P.S. (1995). Purification and mechanisms of action of antitubercular principle from garlic (*Allium sati*6*um*) active against isoniazid susceptible and resistant *Mycobacterium tuberculae* H37RV. *Ind. J. Clinic. Biochem* **10**: 14–18.

Robin, E.H., Anril, W., Alexander, M., Loeto, M. and Keith, K. (1998). Nasopharyngeal carriage and antimicrobial resistance in isolates of *Streptococcus pneumoniae* and *Heamophilus influenzae* Type b in children under 5 years of age in Botswana. *International J. Infect. Dis.* **3(**1): 18–25.

Saxena, K. (1997). Antimicrobial Screening of Selected Medicinal Plants from Ind. *J. Ethnopharm* **58**(2): 75–83.

Saxena, V.K. and Sharma, R.N. (1999). Antimicrobial activity of essential oil of *Lantana aculeata*. *Fitoterapia* **70**(1): 59–60.

Singh, M., Chaudhry, M.A., Yadava, J.N.S. and Sanyal, S.C. (1992). The spectrum of antibiotic resistance in human and veterinary isolates of *Escherichia coli* collected from 1984– 1986 in Northern India. *J. Anti. Chem.* **29**: 159–168.

Tallarida, J.R. (2001). Drug synergism: Its detection and applications. *Pharm* **298**:865-872.

Tomotake, H., Koga, T., Yamato, M., Kassu, A. and Ota, F. (2006). Antimicrobial activity of citrus fruits juices against *Vibrio* species. Iida Women's Junior College, Nagana, Japan, *Nutritional Science Vitamin*. **52(**2): 157-160.

Vishnu, P. S., Mamatha, B., Shivananda, P. G. and Indira, B. (2012). Honey as an antimicrobial agent against Pseudomonas aeruginosa isolated from infected wounds. 4 *J. Global Infect. Dis.* **4**(2): 102-105.

Walsh, C. (2003). Antibiotics: Actions, Origins, Resistance, American Society for Microbiology (ASM) press, Washington , DC.

Winstanley, T.G., Limb, D.L., Eggenton, R. and Hancock, F. (1997). Ten year survey of the antimicrobial susceptibility of urinary tract isolates in the U K: The Microbased Project. *J. Anti. Chem* **40**: 591-594.