



ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES SWEET BASIL (*Ocimum basilicum*) AGAINST DIARRHEA CAUSED BY *Escherichia coli* IN VITRO

¹Muhannad Shweash, ²Atheer Abdulhameed khashan, ³Yasir M. Farhan & ⁴Saddam J. Nasser

¹AL- Anbar University, College of Pharmacy, Clinical Laboratories Sciences Department, Al-Anbar, Ramadi, Iraq

²AL- Anbar University, College of Pharmacy, Pharmacognacy Department, Al-Anbar, Ramadi, Iraq

³AL- Anbar University, College of Pharmacy, Clinical Pharmacy Department, Al-Anbar, Ramadi, Iraq

⁴AL- Anbar University, College of Pharmacy, Pharmaceutics Department, Al-Anbar, Ramadi, Iraq

* Corresponding authors email Dr.muhammadshweash@gmail.com

ABSTRACT

The effects of ethanolic extract of leaves sweet basil (*Ocimum basilicum*) upon enterobacteriaceae *Escherichia coli* in vitro were studied. At a ratio of 100 mg/ml, *Ocimum basilicum* caused a marked increase in zone of inhibition (mm) of the *Escherichia coli* growth. The sizes of inhibition zones were different and substantially increased according to concentration of extract and again the growth was completely inhibited in the highest concentration. A similar outcome was observed using 24 hours incubation period of bacterial growth. Furthermore, *Ocimum basilicum* had dependent concentration effect on *Escherichia coli* inhibition; it extended the diameter of zone inhibition. Induction of zone inhibition was also time dependent. Surprisingly, the results of MIC showed that *Ocimum basilicum* extract had lower MIC (0.312 mg/ml) against *E. coli* which reversal the growth of bacteria mediated by sensitivity to this extract. Unlike outcome was observed using metronidazole, the results demonstrated that *E. coli* was more sensitive to ethanolic extract of *Ocimum* than metronidazole, which was resistance to metronidazole. These data demonstrate for the first time the role of *Ocimum basilicum* extract on diarrhea caused by *Escherichia coli*.

KEYWORDS: Sweet basil (*Ocimum basilicum*), *Escherichia coli*, and Diarrhea

INTRODUCTION

Diarrhea is defined as an increase in the water content, frequency and volume of bowel movement. It can be serious problem, and it was one of the common causes of morbidity and mortality among neonates animals and infants in developing countries (Al-Ukaily, 2009). Survival of the neonatal is imperative for Mild cases of diarrhea disappear within a few days, but sever cases can causes serious dehydration or nutritional problem. The severe dehydration can make the body go into shock and potentially fatal (Rice *et al.*, 2000). The most important cause of diarrhea is *Escherichia coli* which classified in the family *Enterobacteriaceae*, a gram negative, facultative, non-spore forming coccobacilli. Distribution of *E. coli* in the environment is determined by its presence in the bowel of humans and animals. Its presence in water supplies is an indication of recent fecal contamination and the potential presence of enteric pathogens. *E. coli* is easily grown in or on culture media. This microorganism is often referred to as a “coliform bacterium” which is able to ferment lactose with the production of acid and gas (Molenda, 1994 and Wold, 2009).

Resistance in intestinal *E. coli*

Jonathan (2001) has made an experimental attempt to prove that *E. coli* has the ability to become resistant to the antibiotics when having exposure to antimicrobial agents. The development of drug resistance in intestinal bacteria is very different in vitro and in vivo conditions (Yan and Gilbert, 2004). Antimicrobial resistance can be transferred,

rapidly, through a susceptible bacterial population in vitro. The possibility of transfer in the normal gut, however, can be detected only at a very low rate (Licht *et al.*, 1999). Evidence obtained from laboratory and epidemiological studies indicated that the persistence of resistant bacteria was related to the persistence of antimicrobial drug use (Andersson, 2003). If an antimicrobial drug is used, continuously, the persistence of resistant organisms will go on. Thus, *E. coli* has often higher degrees of antimicrobials which have a long history of use (Alhaj *et al.*, 2007). Series of studies on the resistance of *E. coli* which were isolated from animals and humans have strongly suggested that those bacteria which are resistant to antimicrobials used in animals would also be resistant to antimicrobials used in humans (VSPA, 2006; Miles *et al.*, 2006; Umolu *et al.*, 2006). Mayrhofer *et al.*, (2006) showed a direct relationship between the degree of antimicrobial use and resistance in *E. coli* isolates. *E. coli* isolated from different animal species was different concerning the degree of resistance (Burch, 2005). *E. coli* isolates from domestic species was resistant to the largest number of antimicrobial agents tested (neomycin, gentamicin, sulphonamides, chloramphenicol, orfloxacin, tetracycline, ampicillin, cephalothin, Metronidazole, trimethoprim-sulfamethoxazole, nalidixic acid, nitrofurantoin, and sulfisoxazole) compared with isolates from human excretions, wildlife and surface water (Sayah *et al.*, 2005). Antimicrobials which are affected more by the resistance of fecal *E. coli* (Mathew *et al.*, 1999).

Brief history of Medicinal Plant

During the last century, the practice of herbalism became mainstream throughout the world. In spite of great advances observed in modern medicine, plants still make an important contribution to health care. This is due to the recognition of the value of traditional medical systems, particularly of Asian origin, and the identification of medicinal plants from indigenous pharmacopoeias, which have significant healing power. Medicinal plants are distributed worldwide, but they are most abundant in tropical countries (Calixto, 2000; Lewis, 2001).

According to the World Health Organization (WHO, 2001) "a medicinal plant" is any plant which in one or more of its organ contains substances that can be used for the therapeutic purposes or which are precursors for the synthesis of useful drugs. This definition distinguishes those plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to thorough investigation.

The term "herbal drug" determines the part/parts of a plant used for preparing medicines (for example: leaves, flowers, seeds, roots, barks, stems, etc) (Anonymous, 2007).

Ocimum basilicum plant

Classification of *Ocimum basilicum*

Basil (*Ocimum basilicum*) of the family Lamiaceae The plant tastes somewhat like anise, with a strong, pungent, sweet smell. Typically called sweet basil, or Holy basil. The plant grows in several regions around the world. The genus *Ocimum* is ranked high among some of the astonishing herbs for having enormous medicinal potentialities. Previous studies show that there are large numbers of species and varieties falls in this genus (Labra *et al.*, 2004 and Klima'nkova *et al.*, 2008).

Pharmacological Effect of the plant extract (*Ocimum basilicum*)

O. basilicum has also been used in the treatment of a number of ailments like bronchitis, rheumatism and pyrexia(Keita *et al.*, 2000) The fixed oil of *O. basilicum* was found to possess significant anti-inflammatory (Chaurasia *et al.*, 1977) and anti-ulcer activity (Chaurasia *et al.*, 1997), along with anti-microbial (Rana *et al.*, 1997), analgesic and spasmolytic properties without any noticeable toxicity or hypoglycemic effects (Sethi *et al.*, 1979) and is an effective anti-diarrheal, antioxidant, anti-depressant and anti-helmentic and enhances wound healing (Vohora *et al.*, 1973).

In traditional medicine, *Ocimum basilicum* has been used as an antioxidant, antiseptic, preservative, sedative, digestive regulator and diuretic. It also has been recommended for the treatment of headaches, coughs, infections of upper respiratory tract, kidney malfunction and to eliminate toxins (Evans *et al.*, 2006).

O. basilicum belongs to Lamiaceae, distributed worldwide. It is used for various applications as poultice or salve for insect bites, acne and ringworms, as a gargle for mouth or thrush, as a bath herb for increased energy and eye wash for tired eyes. The essential oils of the basil are added to massage for sore muscles. The dried herb used as antiseptic incense and the juice can be applied to fungal infections (Valsara, 1994; Oudhia, 2003).

Basil has been used in Turkish folk medicine for many years and has several therapeutic effects for several conditions such as digestive and appetizing effects (Asimgil, 1997). It was reported that the leafy parts of basil had tonic, antiseptic (Kosekia *et al.*, 2002) and insecticidal properties (Umerie *et al.*, 1998). It is also known the leaves of basil are suitable for the treatment of pain and cough (Basilico and Basilico, 1999). In addition, basil is used for cough treatment, inflammations, dyspepsia, aches and pains (McClatchey, 1996). The essential oil from basil showed an inhibitory effect on *Aspergillus ochraceus* (Basilico and Basilico, 1999) and antimicrobial activity (Hili *et al.*, 1997). The activity of basil against multi-drug resistant clinical isolates from the genera *Staphylococcus*, *Enterococcus* and *Pseudomonas* has been studied (Opalchenova and Obreshkova, 2003).

Ocimum basilicum showed inhibitory effect on *Escherichia coli*, *Klebsiellapneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus sp.* (Durga *et al.*, 2010) the *Ocimum* oil has been described to be active against several species of bacteria and fungi. These include *Listeria monocytogenes*, *Shigella*, *Salmonella* and *Proteus*, for fungi *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Cryptococcus neoformans*, *Penicillium islandicum*, and *Candida albicans* (Lopez *et al.*, 2005)

Ethanol extraction having better inhibition over methanolic extraction. The other extracts indicated an average inhibitory zone diameter for tested pathogenic microorganisms (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*). All antibacterial activities were observed to be concentration dependent.(Tomar U. S. *et al.*, 2010).

MATERIALS & METHODS

Preparation of Crude Organic Solvent Extract of *Ocimum basilicum* Plant

Organic solvent extraction of the *Ocimum basilicum* leaves was carried out by using ethanol (95% ethyl alcohol) which is considered as a very effective in extracting the active ingredients of the plant according to method described by (Effraim, 2000) This was done by using Soxhlet apparatus (Electrothermal company in England), which consists of an electric heater with a thermostat regulator upon which a round bottom glass flask placed that fitted to an extraction unit. The extracting unit contains the solvent and cellulose (thumble) located inside it that contains the dry plant powder. A distiller unit is fitted on to the extraction unit. For condensation of vapor solvent, 50 g. of plant leaves powder was put inside the thumble and 500 ml of 95% ethanol was put inside the flask. The extraction was carried out for 24 hours by heating temperature that kept the solvent at 50-60 C° until a clear and colorless solvent appeared in the extracting unit. After that, the extract was dried by using an electric oven at temperature at 40-45 C° until dry extract was obtained. The dry extract was placed in an incubator under 38-40 C° for complete dryness of the sample .The final extract was kept frozen at -20 C° until use.

Bacteria spp. of Study

Pathogenic bacterial isolate was obtained from the diarrhea patients in Heet Hospital. Bacteria was identified by morphological and biochemical tests.

Analytical Profile Index for *E. coli* Test

It was done by Ministry of Health/Central Public Health Lab. According to Quinn *et al.*, (2004), it was used for

diagnosis of the bacterial spp. By using API 20E system Biomerieux after 24 hrs. incubation at 37 C° as in figure (1)



FIGURE 1: Positive results of *E. coli* on API 20E

In-vitro Antibacterial of Ethanolic Extract of *Ocimum basilicum* Plant

Preparation of Standard Bacterial Suspension

The average number of viable, *Escherichia coli* organism per ml of the stock suspension was determined by means of the Standard McFarland solution No.0.5. By taking 1 ml from overnight culture (nutrient broth) of bacterial suspension washing with 9 ml of Pepton water, then taking 1 ml of this suspension and making serial ten-fold dilution. Standard McFarland solution No.0.5 was prepared according to Baron *et al.*, (1994).

Preparation of Different Concentration of Plant extract

Stock solutions were prepared by mixing 1 g. of dried extract with 10 ml of 50% Dimethylsulphoxide (DMSO) that was sterilized with Millipore membrane filter (0.20µm). Then concentrations of 10, 20, 40, 60, 80 and 100 mg/ml were prepared by mixing known volume from the stock solution with 50% DMSO.

Sensitivity test by using Agar well diffusion method

The agar well diffusion method was adopted according to (Kavanagh, 1972), for assessing the antibacterial activity of the prepared extract. 5 ml of standardized bacterial stock suspensions (1.5×10^8 cfu/ml) of *E. coli* was thoroughly mixed to each 500 ml of sterile Mueller Hinton agar. 20 ml of the inoculated Mueller Hinton agar was distributed into sterile Petri dishes of each. The agar was left to set for 10 minutes to allow solidifying the agar, and in each of these plates 6 well, 6 mm in diameter were cut using a sterile Pasteur pipette and the agar discs were removed by a sterile forceps, after that wells were filled with 0.1ml of each concentration of 10, 20, 40, 60, 80 and 100 mg/ml of *Ocimum basilicum* extract using microtiter-pipette, that allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 C° for 24 hours. Three replicates were carried out for each concentration extract and the activity of plant extract was determined by measuring the diameter

of inhibition zone around each well by millimeter against the tested organism. Simultaneously, addition of the respective solvent (50% DMSO) instead of extract was carried out as controls. The results and standard errors means values were tabulated.

In-vitro Antibacterial of Standard antibiotics

Gentamicin, Amikacin, Metronidazol and Trimethoprim/Sulfamethoxazole were used as a reference antibiotics to determine sensitivity of bacterial species tested (Sharma and Patel, 2009). The same technique which was used for *Ocimum basilicum* antibacterial sensitivity was used for determination of Standard antibiotic activity by using the concentrations of 10, 20, 40, 60, 80 and 100 mcg/ml., 0.1 ml of sterilized distilled water was served as a control.

Determination of Minimum Inhibitory Concentration (MIC) of *Ocimum basilicum* Against Tested Bacteria

MIC was determined by using broth dilution assay method (16). In the tube dilution assay, standard bacterial suspension (1.5×10^8 cfu/ml) was added to tubes containing 10 ml Nutrient broth and different concentration 80mg/ml, 40mg/ml, 20mg/ml, 10mg/ml, 5mg/ml and 2.5mg/ml, 1.25mg/ml, 0.625mg/ml, 0.312mg/ml and 0.156 final concentrations.

Two tubes containing plant extract and nutrient broth served as negative control and positive control, respectively. After 24 h incubation at 37 C°, the tubes were examined for growth. The MIC of extract was taken as the lowest concentration that showed no growth (Asghari *et al.*, 2006; NCCLS, 2000.)

Minimum bactericidal concentration test (MBC) of *Ocimum basilicum* against Tested Bacteria

For minimum bactericidal concentration (MBC) test, (0.1 ml) of broth from test tube containing no growth were plated on to nutrient agar and again incubated overnight at 37 °C overnight. The highest dilution in which no survivor existed was recorded as MBC. (Asghari *et al.*, 2006).

RESULTS & DISCUSSION

Extraction of *Ocimum basilicum*

Extraction of *Ocimum* leaves with 95% ethanol gave a deep green color extract with plant powder yield percentage of 13 % , this was determined by using the following equation:

Percentage yield of the extract = weight of extract (gm) / weight of *Ocimum* powder (gm) × 100 (Banso and Adeyemo, 2006). =13 (gm) / 100 (gm) × 100 =13%

This result is almost similar to the results of Tomar *et al.*, (2010) who found that the percentage recovery of ethanolic extract was 10.6 % w/w from fine *ocimum* leaves powder which was extracted by using a soxhlet apparatus. The near similarity in yield percentage may be attributed to the same solvent which had been used in our extraction.



FIGURE 2: Soxhlet apparatus

In-vitro Antibacterial Activity of Ethanolic Extract of *Ocimum basilicum* Leaves

Different concentrations of ethanolic extract of *Ocimum* were used in agar well diffusion assay, caused different degrees of zones of inhibition against *E.coli*. The sizes of inhibition zones were different according to concentration of extract table (1) and figure (3). The results showed that *E. coli* was more sensitive to ethanolic extract of *Ocimum* than Metronidazole it was resistance to Metronidazole. Dimethylsulfoxide (DMSO) 50% was used as control, it was not give any noticed zone of inhibition, 50% DMSO

was used as a solvent for *Ocimum basilicum* crude extract through both in-vitro and in-vivo studies, it consider one of the solvent that can be used for screening the antimicrobial activity of plant extracts because its 100% biologically inert substances (Fardos, 2009). The results of inhibitory zone indicating the sensitivity of *E. coli* after 24hrs of incubation, towards different tested extract concentrations. All antibacterial activities were observed to be concentration dependent, that was in agreement with Tomar *et al.*, (2010).

TABLE 1: In-vitro antibacterial activity of *O. basilicum* extract in different concentrations on *E. coli* (diameter of inhibition zone in mm)

Con.(mg/ml)	100	80	60	40	20	10
Zone of inhibition (mm)						
Ethanol extract of O.B	24	23	18	15	13	8



FIGURE 3: Sensitivity of *E. coli* to different concentrations of O.B. extract and antibiotics

Determination of Minimum inhibitory concentration (MIC) of *Ocimum Extract* against Bacterial Growth

The results of MIC showed that *Ocimum basilicum* extract had lower MIC (0.312mg/ml) against *E. coli*. Sensitivity of *E. coli* to *Ocimum* extract is in agreement with (Ahmet *et al.*, 2005; Durga *et al.*, 2010) who indicated that, MIC of ethanol extract of *Ocimum* against *E. coli* was below 0.5mg/ml. (Priya *et al.*, 2002) reported that the results of the MIC of extracts on the tested organisms varied widely in the degree of their susceptibility. Antimicrobial agents with low activity against organism have a high MIC while a highly active antimicrobial agent gives a low MIC. Amadioha and Obi, (1999) reported that inactivity of plant extracts may be due to age of plant, extracting solvent, method of extraction and time of harvesting of plant materials. And may due to concentration of solvents, this confirmed our study when we used 95% ethanol for extraction. (Nwinyi, 2009) he referred in his study that ethanol extracts exhibited high inhibitory activity on the test organisms, this can be deduced to the ability of ethanol to extract more of the essential oils and secondary plant metabolites which are believed to exert antibacterial activity on test organisms.

REFERENCES

Ahmet, A., Medine, G. L., Meryem, P.L., Hatice, Ú.T., Fikretin, P., Üsa Karaman (2005) Antimicrobial Effects of *Ocimum basilicum* (Labiatae) Extract. Turk J Biol. 29: 155-160.

Alhaj, N., Mariana, N. S., Raha, A.R., Ishak, Z. (2007) Prevalence of antibiotic resistance among *Escherichia coli* from different sources in Malaysia. *International J. Poult. Sci.* 6 (4), 293-297.

Al-Ukaily, I.A.A. (2009) Effect of Supernatant Antigen of Lactobacillus And Some Plants Extract on immune, Enzyme And Pathological Pictures with *E. coli* Isolated From Children. Ph.D. Thesis. College of Veterinary Medicine-University of Baghdad-Iraq.

Amadioha, A.C., Obi, VI (1999) Control of anthracnose disease of cowpea by *Cymbopogon citrates* and *Ocimum gratissimum*, Acta. Phytopathol. Entomol. Hungarica, 34 (1-2): 85-89.

Andersson, D. I. (2003) Persistence of antibiotic resistant bacteria. *Curr. Opin. Microbiol.* 6 (5), 452-456.

Anonymous (2007) Medicinal, Culinary and Aromatic Plants in the near East-Medi [http://www. faO.Org/dorep/ x5402 e16.htm](http://www.faO.Org/dorep/x5402e16.htm) (cited 4 August, 2007).

Asghari, G. Nourallahi H., Havaie, S. and Issa, L. (2006) Antimicrobial activity of *Otostegia persica Boiss* extracts. RPS; 1:53-58.

Asimgil, A. (1997) Sifali Bitkiler. Timas Yayınları: İstanbul; 101-102.

Banso, A. and Adeyemo, S. (2006) Phytochemical screening and antimicrobial assessment of *Abutilon mauritanum*, *Bacopa monnifera* and *Datura stramonium*. *Biokemistri*, 18(1):39-44.

Baron, E., Peterson, L.R. and Finegold, S. M. (1994) Bailey and Scoffs Diagnostic Microbiology. 9th ed. the C. V Mosby., Co. USA. p. 386-403.

Basilico, M.Z., Basilico, J.C. (1999) Inhibitory effects of some spice essential oils on *Aspergillus ochraceus* NRRL 3174 growth and ochratoxin. A production. *Lett Appl Microbiol* 29: 238-241.

Burch, D. (2005): Problems of antibiotic resistance in pigs in the United Kingdom (<http://www.thepigsite.com/articles/1/health-and-welfare/1266/problems-of-antibiotic-resistance-in-pigs-in-the-uk>, accessed on 16 August, 2007).

Calixto, J.B. (2000) Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicine (Phyto therapeutic agents) *Braz. J. Med Biol. Res* 33:197 – 189.

Chaurasia, S. C. and Vyas, K.K. (1977) In vitro effect of some volatile oil against *Phytophthora parasitica* var. *piperina*. *J. Res. Indian Med. Yoga Homeopath*: 24-26.

Durga K. R., Karthikumar S. Jegatheesan K. (2010) Isolation of potential antibacterial and antioxidant compounds from *Acalypha indica* and *Ocimum basilicum*. *Afr. J. Plant Sci.* Vol. 4(5), pp. 163-166.

Effraim, I.D., Salami, H.A., Osewa, T.S. (2000) The effect of aqueous leaf extract of *Ocimum gratissimum* on haematological and biochemical parameters in rabbits *Afr. J. Biomed. Res.* 175-179.

Evans, W.C., Trease & Evans ``Pharmacognosy``W.B. Saunders and company, reprint 2006, Harcourt and publication. Page no.122

Fardos M. B. (2009) Antifungal activity of some medicinal plants used in Jeddah, Saudi Arabia. *Mycopath*, Vol; 7(1): pp 51-57.

Hili P., Evans, C.S., Veness, R.G. (1997) Antimicrobial action of essential oils: the effect of dimethylsulphoxide on the activity of cinnamon oil. *Lett Appl Microbiol* 24: 269–275.

Jonathan, A. (2001) The development of antibiotic resistance in *Escherichia coli*. (<http://share3.esd105.wednet.edu/mcmillend/02SciProj/jonathana/JonathanA.html>, accessed on 15 August, 2007).

Kavanagh, F. (1972) Analytical Microbiology. F. Kavanagh (ED), Vol: II, Academic press, New York, and London, Pp:11.

Keita, S. M., Vincent C., Schmitt J.P., Belanger A. (2000) Essential oil composition of *Ocimum basilicum* L., *O. gratissimum* L. and *O. suave* L. in the Republic of Guinea. *Flavour Fragr. J.*; 15: 339–341.

Klimaňkova, K. Holadová, J. Hajšlová, T. ajka, J. Poustka and M. Koudela, 2008. Aroma profiles of five basil (*Ocimum basilicum* L.) cultivars grown under conventional and organic conditions. *Food Chem.*, 107: 464-472. doi:10.1016/j.foodchem.2007.07.062.

- Kosekia, P.M., Villavicencio, A.L., Brito, M.S. (2002) Effects of irradiation in medicinal and edible herbs. *Radiat Phys Chem* 63: 681–684.
- Labra, M. Miele, B. Ledda, F. Grassi, M. Mazzei and F. Sala (2004) Morphological characterization, essential oil composition and DNA genotyping of *Ocimum basilicum* L. cultivars. *Plant Sci.*, 167: 725-731. doi:10.1016/j.plantsci.2004.04.026.
- Licht, T.R., Christensen, B.B., Krogfelt, K.A., Molin, S. (1999) Plasmid transfer in the animal intestine and other dynamic bacterial populations: the role of community structure and environment. *Microbiol.* 145, 2615-2622.
- Lopez, P., Sanchez, K., Batlle, R., Nerin, C. (2005) Solid and vapour phase anti-microbial activities of six essential oils susceptibility of selected food borne bacterial and fungal strains. *J. Agric Food Chem.* 53(17): 6939-6946.
- Mathew, A.G., Saxton, A.M., Upchurch, W.G., Chattin, S.E. (1999) Multiple antibiotic resistance patterns of *Escherichia coli* isolates from swine farms. *Appl. Environ. Microbiol.* 65 (6), 2770-2772.
- Mayrhofer, S., Paulsen, P., Smulders, F.J.M., Hilbert, F. (2006) Short Communication: Antimicrobial resistance in commensal *Escherichia coli* isolated from muscle foods as related to the veterinary use of antimicrobial agents in food-producing animals in Austria. *Microbial Drug Resistance.* 12 (4), 278 -283
- McClatchey W. (1996) The ethnopharmacopoeia of Rotuma. *J Ethnopharmacol* 50: 47–156.
- Miles, T. D., McLaughlin, W., Brown, P.D. (2006) Antimicrobial resistance of *Escherichia coli* isolates from broiler chickens and humans. *BMC Vet. Res.* 2:7.
- Molenda, J. R. (1994) *Escherichia coli* (Including O157:H7): environmental health perspective. *Dairy Food and Environmental Sanitation*, 14(12):742-747.
- NCCLS (2000) National Committee for Clinical Laboratory Standards. Approved Standard M7-A5: Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically, 5th Ed.
- Nwinyi, O. C., Chinedu, N. S., Ajani, O. O., Ikpo C. O. and Ogunniran, K. O. (2009) Antibacterial effects of extracts of *Ocimum gratissimum* and *piper guineense* on *Escherichia coli* and *Staphylococcus aureus*. *Afr. J. Food Sci.* Vol. 3(3). pp. 077-081
- Opalchenova, G., Obreshkova, D. (2003) Comparative studies on the activity of basil – an essential oil from *Ocimum basilicum* L. against multidrug resistant clinical isolates of the genera *Staphylococcus*, *Enterococcus* and *Pseudomonas* by using different test methods. *J Microbiol Methods* 54: 105–110.
- Oudhia, P. (2003) Traditional medicinal uses in India. *J. Planta Medica.* 15(5): 175-179.
- Priya, K.S., Gnanamani, K.A., Radhakrishnan, N. and Baba, M. (2002) Healing product on *Datura alba* on burn wound in albino rats. *Journal of Ethnopharmacology*, 83193-199.
- Quinn, P. J., Carter, M.E., Markey, B. and Carter, G.R (2004) *Clinical Veterinary Microbiology*. Mosby. New York. USA. Pp: 26-63.
- Rana, B. K., Singh, U. P. and Taneja, V. (1997) Antifungal activity and kinetics of inhibition by essential oil isolated from leaves of *Aegle marmelos*. *J. Ethnopharmacol.*; 57:29-3.
- Rice, A.L., Sacco, R.D., Hyder, J. and DreBloack, N. (2000) Malnutrition as an underlying cause of childhood deaths associated with infection diseases in developing countries, 78:1207-1221.
- Sayah, R.S., Kaneene, J. B., Johnson, Y., Miller, R.A. (2005) Patterns of antimicrobial resistance observed in *Escherichia coli* isolates obtained from domestic- and wild-animal fecal samples, human septage, and surface water. *Appl. Environ. Microbiol.* 71 (3), 1394–1404.
- Sethi M. L. (1979) Inhibition of reverse transcriptase activity by benzophenanthridine alkaloids. *J. Nat. Prod.*; 42:187-196.
- Vohora S. B., M. Rizwan and J. A. (1973). *Khan Medicinal uses of common Indian vegetables*. *Planta Med.*; 23:381-393
- Sharma, A. and Patel, V.K. (2009) In-vitro screening of the antibacterial activity and identification of bioactive compounds from plants against selected *Vibrio* spp. *Pathogens. Turk. J. Biol.*, 33:137-144.
- Tomar, U.S., Daniel, V., Shrivastava, K., Panwar Mangal, S. and Pant, P. (2010) Comparative evaluation and antimicrobial activity of *Ocimum basilicum* Linn. (Labiatae). *J. Gl. Pha. Tech.*; 2(5): 49-53
- Umerie, S.C., Anaso, H.U., Anyasoro, L.J.C. (1998) Insecticidal potentials of *Ocimum basilicum* leaf-extract. *Bioresourch Technol* 64: 237–239.
- Umolu, P.I., Ohenhen, E.R., Okwu, I.G., Ogiehor, I. S. (2006) Multiple Antibiotic resistant index and plasmid of *Escherichia coli* in beef in Ekpoma. *Am. J. Sci.* 2 (3), 1-4
- Valsara, T. (1994) Screening of Indian Medicinal plants for antimicrobial activity. *J. Ethnobiol.* 35(3): 275-283.
- VSPA (World Society for the Protection of Animals) (2006): Resistant bacteria in people and farm animals around the world. (<http://wspafarmwelfare.org/>) hantibiotics. html, accessed on 15 August, 2007).
- WHO (2001) *Legal Status of Traditional Medicine and Complementary/ Alternative medicine: A world wide review*. WHO Publishing 1.
- Wold, A. E. & Adlerberth, I. (2009) Establishment of the gut microbiota in Western infants. *Acta Paediatr* 98, 229-238.
- Yan, S.S., Gilbert, J.M. (2004) Antimicrobial drug delivery in food animals and microbial food safety concerns: An overview of in vitro and in vivo factors potentially affecting the animal gut micro flora. *Adv. Drug. Deliv. Rev.* 56 (10), 1497-1521.