Northeast Journal of Contemporary Research, December 2019. Vol. 6 No. 1, pp 1-9
ISSN 2349-3089 ©NeJCR, 2019

Antibiotic sensitivity pattern of some enteric pathogens collected from Tezpur, Assam and antimicrobial activity of ethanolic extract of *Scoparia dulcis* L.

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ABSTRACT

Diarrhoea remains a major problem in the South East Asian countries, including India. There is therefore, a need to understand the nature of antibiotic resistance patterns of various enteric pathogens causing diarrhoea. The investigation was conducted to understand the antibacterial activity of certain drugs presently in use for the treatment of enteric disease with its status of resistant, intermediate or sensitive. Study was also conducted to understand the efficacy of plant extracts as many of the drugs presently in use have grown resistant and have probable side effects. A total of 54 stool samples were collected from children upto six years of age from the children ward of Kanaklata Civil Hospital, Tezpur and from the nearby villages of Gutlong, Panchmile and Napaam, Assam, India. The plant material tested was the leaf part of *Scoparia dulcis* Linn. The bacterial strains showed multidrug resistance. The MAR was found to range from 31% to 68%. The findings indicate that the clinical isolates of the enteric pathogens are multidrug resistant and alternative drugs need to be designed for better management of the disease. Ethanolic extract of the leaves of *Scoparia dulcis* L. showed an inhibition zone of 11mm with 10 µl plant extract, 16 mm with 20 micro µl plant extract and 17 mm with 30 µl plant extract. Moreover, phytochemical analysis conducted on the plant extracts revealed the presence of phytochemicals such as flavinoids, glycoside, cardiac glycoside, steroid, tannins, alkaloids, phytosterol and terpenoids. The leaf extracts of *Scoparia dulcis* L. can therefore be used for better drug designing.

Key words: Diarrhoea, enteric pathogens, antibiotic resistance, MAR index, phytochemical screening

INTRODUCTION

Infectious diarrhoeal diseases are responsible for considerable morbidity and mortality, especially in developing countries (Guerrant *et al.*, 1990). Resistance has emerged even to newer, more potent antimicrobial agents and is commonly seen in organisms like *Salmonella, Shigella* and *Vibrio cholerae* (Sack *et al*, 1997; Replogle *et al.*, 2000; Hoge *et al.*, 1998; Threlfall *et al.*, 1997; Jiang *et al.*, 2002; Niyogi *et al.*, 1999; Jesudason, 2002; Chunder *et al.*, 1997; Kain *et al.*, 1991). In one study it was found that the *V. cholerae* isolates were generally susceptible to tetracycline. Intermediate level of resistance to ciprofloxacin was also reported (Garg *et al.*, 2010).

Bacteria have the genetic ability to

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transmit and acquire resistance to drugs which are utilized as therapeutic agents. The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. Therefore, there is a need to search new infection fighting strategies to control microbial infections (Sieradzki *et. al.*, 1999). There is a great need to reduce the indiscriminate use these potent antibiotics, the drug of choice for serious endemic infections like enteric fever. There is a need to educate both the general public and the health practitioners that most diarrhoea does not require antibiotics. It is also necessary to carry out periodic monitoring of drug resistance in enteropathogens in different geographic areas so that an appropriate agent can be chosen for empiric therapy (Mukhopadhyay and Sur, 1996; Taneja *et al.*, 2014).

Due to the cost effectiveness, safety, increasing failure of chemotherapy and antibiotic resistance, search for plant resources has been increased for their potential antimicrobial activity (Hammer *et al.*, 1999). Medicinal plants are used locally in the treatment of infections caused by fungi, bacteria, viruses and parasites. Different plants have been used as a source of inspiration in the development of novel drugs. Plant derived medicines are widely used because they are relatively safer than the synthetic alternatives and they are easily available and cheaper. Many plant species have been evaluated for their antimicrobial activity in the past 20 years. The active components of many drugs found in plants are secondary metabolites (Robbers *et al.*, 2016).

The use of plants for treating diseases is as old as the human species. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as communicable diseases (Saleh *et al.*, 2009). Plants are the richest source of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al.*, 1999). It is Ayurveda, the foundation of the medicinal science of Hindu culture, in its eighth division deals with specific properties of drugs and various aspects of science of life and the art of healing (Rastogi & Mehrotra, 2002). Among those antibacterial foods that are becoming more common in western diet are green tea and ginger (Sharma *et al.*, 2009). The use of plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. The active compounds of many drugs found in plants are secondary metabolites. The identification and isolation of such active compounds makes it more effective in therapeutic application (Sathyadevi *et al.*, 2014).

Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. The objective of this research work was to evaluate the antibiotic resistance pattern of some enteric pathogens and to determine the antibacterial activity of the leaf extracts of *Scoparia dulcis* L.

MATERIALS AND METHODS

A total of 54 pathogens were isolated between 2008 and 2010 from the stool samples collected from the patients admitted to Kanaklata Civil Hospital, Tezpur, Assam and the nearby villages of Gutlong, Panchmile and Napaam. The antibiotic disks required for the sensitivity test were procured from Himedia, Mumbai, India. The leaves of the plant (*Scoparia dulcis* L.) required were collected locally from Tezpur during December, 2016 and identified following standard literature (Sharma *et al.*, 2009). The leaves were washed and shade dried for extraction of the active components.

Bacterial identification

The microorganisms isolated are identified by standard biochemical methods as stated

in the Bargey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

Antimicrobial susceptibility test

The antimicrobial susceptibility was determined according to Kirby and Bauer disk diffusion method using commercially available antimicrobial discs (Hi-Media Laboratories, Mumbai, India). The nutrient medium Mueller-Hinton agar with a pH of 7.2 to 7.4 was poured into plates to a uniform depth of 5mm and refrigerated on solidification. Prior to use, the plates are transferred to an incubator at 37°C for 10 to 20 minutes to dry of the moisture that develops on the agar surface. The plates were then heavily inoculated (500µlt per plate) to ensure the confluent growth of organisms. The antibiotic discs were then aseptically applied on the surface of the agar plate at well spaced intervals. Once applied, each disc is gently touched with a sterile applicator stick to ensure its firm contact with the agar surface. Following overnight incubation, the plates are examined for the presence of inhibition of bacterial growth which was indicated by a clear zone surrounding each disc. The susceptibility of an organism to a drug is determined by the size of this zone.

The measurement of the diameter of the zone of inhibition was done in millimeters and its size was compared to that contained in a standardized chart. Based on this comparison, the test organism was determined to be resistant, intermediate, or susceptible to the antibiotic. The antibiotic sensitivity test was carried out using the antibiotic discs of Kanamycin (30 mcg), Nalidixic acid (30 mcg), Neomycin (30 mcg), Norfloxacin (10 mcg), Rifampicin (5 mcg), Tetracycline (10 mcg), Streptomycin (10 mcg), Novobiocin (5 mcg), Gentamicin (10 mcg), Erythromycin (15 mcg), Cotrimazine (25 mcg), Furazolidone (50 mcg), Ampicillin (10 mcg), Chloramphenicol (30 mcg), Ciprofloxacin (10 mcg), Cinoxacin (10 mcg). The MAR (multiple antibiotic resistance) index (Krumperman, 1985) was then calculated (Bauer *et. al.*, 1996).

Extraction of plant material and test for antibacterial activity

The plant materials were obtained locally from Tezpur. It was then shade dried, powdered and subsequently subjected to the extraction process (Kambizi and Afolayan, 2001). The extraction was done by a Soxhlet apparatus using ethanol as a solvent. The plant material tested were the leaf parts of *Scoparia dulcis* L. To the powdered plant material ethanol was added and incubated with stirring for 24 hours. Soxhlet extraction of the plant powders was also subsequently done. The filtered extracts were then evaporated to dryness and re-dissolved in ethanol to obtain the required concentration. The extracts were passed through a filter of 0.2 micron porosity (Sartorius, Germany) and then kept at –4°C till use.

Filter paper discs (Whatmann paper no.1) were sterilized by autoclaving and the extract was added onto each disc to obtain concentrations of 10 µl, 20 µl, and 30µl per disc. The discs thus prepared were dried before use. Culture for drug sensitivity was carried out on Nutrient agar (Himedia, Mumbai) upon prior subculture on Nutrient broth. Fresh cultures of the bacterial pathogens were used for testing the plant activity. The antibacterial activity of the plant extract was evaluated using the disc diffusion method. The disc inhibition zone was evaluated by using a modification of the method described earlier (Bauer *et. al.*, 1996).

Phytochemical analysis:

The freshly prepared extracts were chemically tested for the presence of different phytochemical constituents such as alkaloids, flavonoids, phenolic compounds, steroids, saponins, tannins, etc. by using standard methods (Khandelwal, 2004).

RESULTS & DISCUSSION

From the 54 stool samples collected and studied 28 species of *Escherichia coli*, 15 species of *Shigella*, and 11 species of *Salmonella* were identified by standard biochemical methods discussed earlier. The strains showed multidrug resistance. The pathogens showed resistance towards Kanamycin, Nalidixic acid, Rifampicin, Novobiocin, Erythromycin, Ampicillin, Vancomycin and Cinoxacin. However, most the strains were sensitive to Neomycin, Norfloxacin, Streptomycin, Gentamycin, Chloramphenicol and Ciprofloxacin (Table-1). Table-1 also shows the antibiogram of the pathogens with different antibiotics. The MAR index was found to range between 31% and 68%. Different antimicrobial have become widely available and the capricious use of antibiotics has led to the rapid emergence of bacterial resistance, with a greater infection burden for vulnerable individuals. Many studies have shown a dramatic shift in antibiotic resistance among pathogenic bacteria, which has led to the swift spread of many infectious diseases (Ries *et al.*, 1994; Threlfall *et al.*, 1992; Weber *et al.*, 1994; Yamamato *et al.*, 1995; Sebiomo *et al.*, 2011).

It was found that ethanolic extract of the leaves of *Scoparia dulcis* L. showed an inhibition zone of 11mm with 10 µl plant extract, 16 mm with 20 micro µl plant extract and 17 mm with 30 µl plant extract (Plate 1). The control experiment containing ethanol with no plant extract showed no bacterial growth inhibition or antibacterial activity.

Indian medicinal plants are used in various system of medicine because of its minimal side effects and cost effectiveness (Irfan & Atiya, 2004). The currently available antibiotics like ceftazidime, chloramphenicol and co-trimoxazole have been documented to be potentially toxic and possess harmful side effects (Suputtamongkol *et al.*, 1991). Chloremphenicol has predictable haemopoietic suppression and aplastic anaemia.

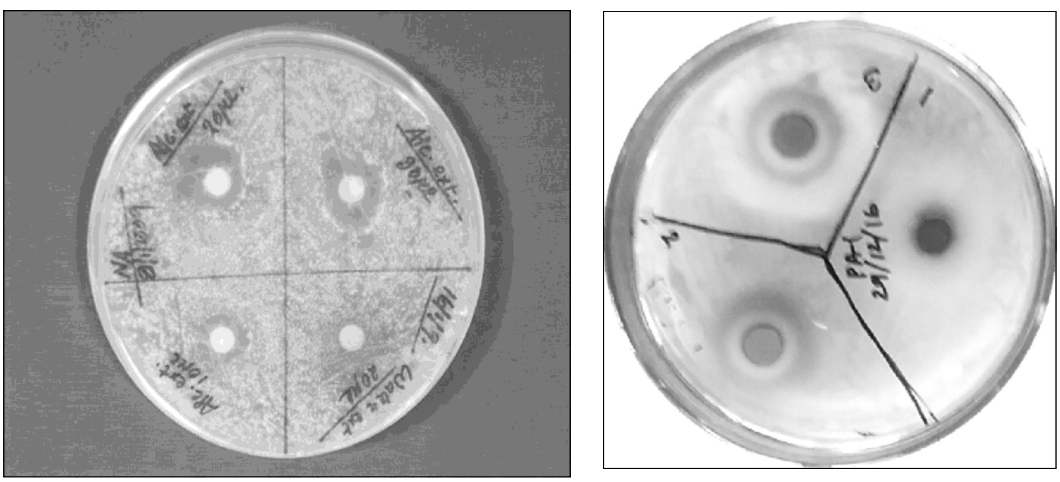

Plate 1. Culture plates showing antibacterial activity of *Scoparia dulcis* L. at various concentrations

The results showed the antibacterial activity of leaf parts of *Scoparia dulcis* L. Indian medicinal plants are regularly used in various systems of medicine because of minimal side effects and cost-effectiveness (Muthu *et al.*, 2005). Further attempts may be focused on finding out the active component from the leaves of *Scoparia dulcis* L. which could be an important source of new antimicrobial agent against the enteric pathogens.

In the present investigation it has been found that that the MAR index ranges from 0.31 – 0.68, which indicates that the enteric pathogens collected, identified and studied are resistant to 31% to 68% of the antibiotics taken in the study. However, all the strains are sensitive to chloramphenicol. This clearly depicts that the strains are multi-drug resistant and new works are to be focused on developing new antibiotics with more efficacy.

The phytochemical analysis conducted on the plant extracts revealed the presence of phytochemicals such as flavinoids, glycoside, cardiac glycoside, steroid, tannins, alkaloids, phytosterol and terpenoids (Table 2). Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls.

The currently available antibiotics such as chloramphenicol and co-trimoxazole have been documented to be potentially toxic and possess harmful side effects (Suputtamongkol *et al.*, 1991). Plant extracts can provide effective medicines and they are also cost effective with no side effects. In the present study the ethanolic extracts of the leaves of *Scoparia dulcis* L. showed good bacterial inhibition zones. The MIC (minimum inhibitory concentration) measurement study indicates that these plant extracts can be used in developing effective medicines against diarrhoeal diseases. However, the works on plant extracts has been done with crude extracts only and therefore efforts will be focused on purifying these extracts by column chromatography, HPLC and other biochemical techniques in further studies and thus finding out the active component responsible for the zone of inhibition.

Table 1. Shows the antibiotic resistance pattern of different bacterial strains and the MAR index

Culture no.	Resistance pattern	MAR index
AJ1	K, Na, R, T, No, E, A, Cx	0.50
AJ2	Na, R, T, S, No, G, E, Co, Cx	0.56
AJ3	Na, Ne, R, T, No, G, E, Co, A, Ci, Cx	0.68
AJ4	Na, N, R, T, S, No, Co, Ci, Cx	0.56
AJ5	Na, R, T, No, E, Cx	0.37
AJ6	Na, R, T, No, E, Ci, Cx	0.43
AJ7	Na, R, T, No, E, Cx	0.37
AJ8	K, Na, R, T, No, E, Cx	0.43
AJ9	Na, R, T, No, G, Cx	0.38
AJ10	Na, R, T, No, Cx	**0.31**
AJ11	K, Na, R, T, S, No, Co, A, Cx	0.56
AJ12	K, Na, R, T, No, E, Cx	0.43
AJ13	Na, R, T, No, E, R	0.38

AJ14	Na, R, T, No, G, E, Cx	0.43
AJ15	K, Na, R, T, No, E, Co, A, Cx	0.56
AJ16	Na, R, T, S, No, F, A, Cx	0.50
AJ17	Na, R, T, No, E, F, A, Cx	0.50
AJ18	K, Na, R, T, No, G, F, Cx	0.50
AJ19	Na, R, T, No, E, Co, Cx	0.43
AJ20	Na, R, T, No, E, Cx	0.38
AJ21	K, Na, R, No, E, Co, Cx	0.43
AJ22	Na, R, T, No, G, E, A, Cx	0.50
AJ23	Na, R, T, No, E, A, R	0.43
AJ24	K, Na, R, T, No, E, Co, Cx	0.50
AJ25	Na, Ne, R, T, S, No, G, E, Cx	0.56
AJ26	Na, R, T, S, No, G, E, Co, Ci, Cx	0.62
AJ27	K, Na, R, T, No, G, E, Co, A, Ci, Cx	**0.68**
AJ28	Na, R, T, No, E, Co, A, Cx	0.50
AJ29	K, Na, Ne, R, T, No, E, Cx	0.50
AJ30	Na, R, T, S, No, G, E, Co, Cx	0.56
AJ31	K, Na, R, T, No, G, E, Ci, Cx	0.56
AJ32	Na, R, T, No, G, Co, A, Ci	0.50
AJ33	Na, R, T, S, No, G, E, Cx	0.50
AJ34	K, Na, R, T, No, F, Ci, Cx	0.50
AJ35	K, Na, Ne, R, T, No, G, E, Co, Cx	0.62
AJ36	Na, R, T, No, G, E, Co, Ci, Cx	0.56
AJ37	Na, R, T, No, Co, F, Ci, Cx	0.50
AJ38	Na, R, T, S, No, Co, Cx	0.43
AJ39	K, Na, R, T, Co, A, Ci, Cx	0.50
AJ40	Na, R, T, No, G, E, Cx	0.43
AJ41	Na, R, T, No, E, F, Ci	0.43
AJ42	Na, Ne, R, T, No, E, Co, Cx	0.50
AJ43	Na, R, T, No, G, Cx	0.37
AJ44	Na, R, T, S, No, E, Co, Ci, Cx	0.56
AJ45	Na, R, T, No, Co, A, Cx	0.43
AJ46	Na, R, T, No, G, Cx	0.37
AJ47	K, Na, R, T, No, G, Co, Cx	0.50
AJ48	K, Na, R, T, No, G, Co, Cx	0.50
AJ49	Na, Ne, R, T, No, A, Ci, Cx	0.50
AJ50	K, Na, R, T, S, No, G, Ci, Cx	0.56
AJ51	Na, R, T, S, No, E, F, A, Ci, Cx	0.62
AJ52	Na, R, T, No, E, Co, F, Cx	0.50
AJ53	Na, Ne, R, T, No, G, Co, Ci, Cx	0.56
AJ54	Na, R, T, No, Cx	0.31

Table 2. Phytochemical screening of *Scoparia dulcis* L.

Phytochemical constituents	Aqueousextract	Ethanolicextract	Acetone extract	$DMSO_4$ extract
Flavonoid	-	+	+	+
Glycoside	-	+	+	+
Cardiac glycoside	-	+	+	+
Steroid	-	+	-	+
Tannins	+	+	+	+
Alkaloids	-	+	+	+
Phytosterol	-	-	+	+
Terpenoids	-	+	-	+

K, Kanamycin (30mcg); Na, Nalidixic acid (30mcg); Ne, Neomycin (30mcg); N, Norfloxacin (10mcg); R, Rifampicin (5mcg); T, Tetracycline (10mcg); S, Streptomycin (10mcg); No, Novobiocin (5mcg); G, Gentamicin (10mcg); E, Erythromycin (15mcg); Co, Cotrimazine (25mcg); F, Furazolidone (50mcg); A, Ampicillin (10mcg); Ci, Ciprofloxacin (10mcg); Cx, Cinoxacin (10mcg); MAR, multiple antibiotic resistance.

CONCLUSION

In the present investigation it has been found that that the MAR index ranges from 0.31 – 0.68, which indicates that the enteric pathogens collected, identified and studied are resistant to 31% to 68% of the antibiotics taken in the study. However, all the strains are sensitive to chloramphenicol. This clearly depicts that the strains are multi-drug resistant and new works are to be focused on developing new antibiotics with more efficacy. The currently available antibiotics such as chloramphenicol and co-trimoxazole have been documented to be potentially toxic and possess harmful side effects. Plant extracts can provide effective medicines and they are also cost effective with no side effects. Based on the present investigations it can be concluded that the plant can be a potential source for herbal drug against the human pathogenic bacteria. In the present study the ethanolic extracts of the leaves of *Scoparia dulcis* L. showed good bacterial inhibition zones. The MIC (minimum inhibitory concentration) measurement study indicates that these plant extracts can be used in developing effective medicines against diarrhoeal diseases. However the works on plant extracts has been done with crude extracts only and therefore effort need to be focused on purifying these extracts by column chromatography, HPLC and other biochemical techniques and thus find out the active component responsible for the zone of inhibition.

REFERENCES

Bauer A.W., Kirby W.W.M., Sterris J.C., and Turck M. 1996. Antibiotic susceptibility testing by a standard single disc method. *American Journal of Clinical Pathology*, 45: 493 – 496.

Chunder N., Bhattacharya S.K., Biswas D., Niyogi S.K., and Kumar R. 1997. Isolation of a fluoroquinolone resistant *Shigella dysenteriae* 1 strain from Calcutta. *Indian Journal of Medical Research,* 106: 494-496.

Garg P., Chakroborty S., Basu I., Datta S., Rajendran K., Bhattachyarya T., Yamasaki T., Bhattacharya S.K., Takeda Y., Nair G.B., and Ramamurthy T. 2010. Expanding multiple antibiotic resistance among clinical strains of *Vibrio cholerae* isolated from 1992-97 in Calcutta, India. *Epidemiology and Infection,* 124: 393-399.

Hammer K.A., Carson C.F., and Riley T.V. 1999. Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology,* 86(6): 985.

Hoge C.W., Gambel J.M., Srijan A., Pitarangsi C., and Echeverria P. 1998. Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. *Clinical Infectious Diseases,* 26: 341-345.

Holt J.C., Krieg N.R., Sneath P.H., Staley J.T., and Williams S.T. 1994. Bergey's Manual of Determinative Bacteriology, Williams and Wilkins Company, Baltimore, USA.

Irfan A.K., and Atiya K. 2004. A panaroma of ethnobotany. In : Ethnomedicine and human welfare, Ukaaz Publishers, Hyderabad, India.

Jesudasan M.V. 2002. *Shigella* isolation in Vellore, South India (1997—2001). *Indian Journal of Medical Research,* 115: 11 – 13.

Jiang Z.D., Lowe B., Verenkar M.P., Ashley D., Steffen R., and Tornieporth N. 2002. Prevalence of enteric pathogens among international travelers with diarrhea acquired in Kenya (Mombasa), India (Goa), or Jamaica (Montego Bay). *Journal of Infectious Diseases,* 185: 497-502.

Kain K.C., Barteluk R.L., Kelly M.T., He X., de Hua G., and Ge Y.A. 1991. Etiology of childhood diarrhea in Beijing, China. *Journal of Clinical Microbiology,* 29: 90 -95.

Kambizi L., and Afolayan A.J. 2001. An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve district, Zimbabwe. *Journal of Ethnopharmacology,* 71: 5-9.

Khandelwal K.R. 2004. Practical Pharmacognosy, Nirali Prakshan, Pune, India, 149-156.

Krumperman P.H. 1985. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Applied Environmental Microbiology,* 46: 165 – 170.

Mukhopadhyay S.P., and Sur D. 1996. Case study to strengthen health sector preparedness and response in flood prone districts in West Bengal. *Journal of Communicable Diseases,* 7: 11—17.

Muthu S.E., Nandakuma S., and Rao U.A. 2005. The effect of methanolic extract of *Tamarindus indica* Linn. on the growth of clinical isolates of *Burkholderia pseudomallei*. *Indian Journal of Medical Research,* 122: 525 – 528.

Niyogi S.K., Dutta D., Bhattacharya M.K., and Bhattacharya S.K. 1999. Multi-drug resistant non-typhoidal *Salmonella* spp. associated with acute diarrhoeal disease. *Indian Journal of Medical Research,* 110: 183-185.

Rastogi R.P., and Mehrotra B.N. 2002. Glossary of Indian Medicinal Plants, National Institute of science communication, New Delhi, India.

Replogle M.L., Fleming D.W., and Cieslak P.R. 2000. Emergence of antimicrobial-resistant Shigellosis in Oregon. *Clinical Infectious Diseases*, 30: 515-519.

Ries A.A., Wells J.G., Olivola D., Ntakibirora M., Nyandwi S., and Ntibakivayo M. 1994. Epidemic *Shigella dysenteriae* type I in Burundi: panresistance and implications for prevention. *Journal of Infectious Diseases*, 169: 1035 – 1041.

Robbers J., Speedie M., and Tyler V. 1996. Pharmacognosy and Pharmacobiotechnology. Williams and Wilkins, Baltimore, 1-14.

Sack R.B., Rahman M., Yunus M., and Khan E.H. 1997. Antimicrobial resistance in organisms causing diarrheal disease. *Clinical Infectious Diseases,* 24 (1): 102-105.

Saleh A.I., Mohammad Al-Dosari S., Abdul M., Alsheikh M., and Abdel-Kader S. 2009. Evaluation of the hepatoprotective effect of *Fumaria parviflora* and *Momordica balsamina* from Saudi folk Medicine against experimentally induced liver injury in rats. *Research Journal of Medicinal Plants*, 3(1): 9-15.

Sathyadevi M., Suchithra E.R., and Subramanian S. 2014. *Physalis peruviana* Linn. fruit extract improves insulin sensitivity and ameliorates hyperglycemia in high-fat diet low dose STZ-induced type 2 diabetic rats. *Journal of Pharmaceutical Research*, 8(4): 625-632.

Sebiomo A., Awofodu A.O., Awosanya F.E.A., and Ajayi A.J. 2011. Comparative studies of antibacterial effect of some antibiotics and ginger (*Zingiber officinale*) on two pathogenic bacteria. *Journal of Microbiology and Antimicrobials,* 3(1): 18-22.

Sharma U., Saini R., Kumar N., and Singh B. 2009. Steroidal saponins from *Asparagus racemosus*. *Chemical & Pharmaceutical Bulletin,* 57(8): 890-893.

Sieradzki K., Wu S.W., and Tomasz A. 1999. Inactivation of the methicillin resistance gene mecA in vancomycin resistant *Staphylococcus aureus*. *Microbial Drug Resistance*, 5(4): 253-257.

Suputtamongkol Y., Dance D.A., Chaowagul W., Wattanagoon Y., Wuthiekanun V., and White N.J. 1991. Amoxycillin-clavulanic acid treatment of melioidosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 85: 672—675.

Taneja N., Kaur J., Sharma K., Singh M., Kalra J.K., and Sharma N.M. 2014. A recent outbreak of cholera due to *Vibrio cholerae* 01 Ogawa in and around Chandigarh, North India. *Indian Journal of Medical Research,* 117: 243 – 246.

Threlfall E.J., Graham A., Cheasty T., Ward L.R., and Rowe B. 1997. Resistance to ciprofloxacin in pathogenic enterobacteriaceae in England and Wales in 1996. *Journal of Clinical Pathology,* 50: 1027-1028.

Threlfall E.J., Ward L.R., Rowe B., Raghupati S., Chandrasekaran V., and Vandepitte J. 1992. Widespread occurrence of multidrug resistant *Salmonella typhi* in India. *European Journal of Clinical Microbiology and Infectious Diseases*, 11: 990 – 993.

Weber J.T., Mintz E.D., Canizares R., Semiglia A., Gomez I., and Sempertegui R. 1994. Epidemic cholera in Ecuador: multidrug resistance and transmission by water and sea food. *Epidemiology and Infection*, 112: 1 –11.

Yamamoto T., Nair G.B., and Takeda Y. 1995. Emergence of tetracycline resistance due to a multi-drug resistance plasmid in *Vibrio cholerae* O139. *FEMS Immunology and Medical Microbiol*ogy, 11: 131 – 136.