INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 16, No. 2, p. 31-39, 2020

RESEARCH PAPER OPEN ACCESS

In-vitro antibacterial interaction of crude methanol extract of *Zingiber officinale* with a quinolone against *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis*

Clement Oliseloke Anie, Michael Oghenejobo*, Esther Imabong Edomokong, Ejemighaye, Tracy

Pharmaceutical Microbiology Department, Faculty of Pharmacy Delta State University Abraka Nigeria

Key words: Ciprofloxacin, *Zingiber officinale, Bacillus subtilis, Staphylococcus aureus Escherichia coli.*

http://dx.doi.org/10.12692/ijb/16.2.31-39 Article published on February 05, 2020

Abstract

The aim of this study is to determine the *in-vitro* antibacterial interaction of crude methanol extract of *Zingiberofficinale* with Ciprofloxacin, the remedial implications of the simultaneous use of *Zingiberofficinale*(herbal remedy) and Ciprofloxacin (conventional medicine). The plant material was obtained, peeled, dried, ground and then extracted using cold maceration method with 80% methanol. Phytochemical screening was conducted. Antibacterial activity of methanol extracts of *Zingiberofficinale* at various concentrations was done using the agar well diffusion method with Ciprofloxacin used as the positive control while Methanol was used as negative control. Minimum inhibitory concentrations (MIC) of *Zingiber officinale* and Ciprofloxacin were evaluated using *Bacillus subtilis, Escherichia coli and Staphylococcus aureus* as test organisms. Then the synergistic effect of the methanol extracts of *Zingiberofficinale* rhizome and Ciprofloxacin was also carried out. Phytochemical result revealed the presence of saponins, steroid, terpenoids, alkaloids and flavonoids. Antimicrobial evaluations showed zones of inhibition against the three test organisms (*E. coli, S. aureus,* and *B. subtili*), ranging from 1mm to 7.5mm at the various concentrations for the methanol extract of *Zingiberofficinale*. The zone of inhibition of the positive control (Ciprofloxacin) was observed to be from 39.5 to 35mm. The minimum inhibitory concentration (mg/ml) was observed to be 800, 50 and 50 for *E. coli, B. subtilis*, and *S. aureus* respectively for the methanol extracts of *Zingiber officinale* and 0.25, 0.5, 0.125 for *S. aureus, E. coli,* and *B. subtilis* respectively for Ciprofloxacin.The synergistic effect of the methanol extracts of *Zingiber officinale* and Ciprofloxacin was seen to have a higher zone of inhibition (26 mm) against *Staphylococcus aureus*, unlike *Escherichia coli* and *Bacillus subtilis*, that showed antagonism when the extract and ciprofloxacin were combined. is study confirms that *Zingiberofficinale* possesses antimicrobial activity, and the combined effect of the interaction between *Zingiberofficinale* and Ciprofloxacin showed a synergistic activity against *Staphylococcus aureus*.

* **Corresponding Author:** Michael Oghenejobo ✉ jobomic1@yahoo.com

Introduction

Humans have made several efforts to remedy the prevalence of many diseases situation in order to recover a life uninterrupted by these ailments. The original type of healing substances had always been the herbal medicines, however with the coming of civilization that has led to better scientific appreciative of diseases and their medications, conventional medicines have turned into the major and really known materials for disease management in current health system (Osemene *et al.*, 2011). Herbal medicines are defined as herbal preparations, even finished herbal products that contain different parts of plant as active the ingredients (Mahomoodally, 2013). Drugs on the other side are known to be pure chemical compounds which when they are administered into the human bodies to produce pharmacological effects that may accordingly result to lessening of the disease or be helpful in the prevention or diagnosis of disorders (Chan and Cheung, 2000). So many modern conventional drugs have had their herbal origin, although the major difference between herbal and orthodox medicines is that the later contains more number of compounds, rather than single pharmacologically active substance; therefore, different components of both orthodox and herbal medicines can act on one another to enhance an effect or oppose (Houghton, 2009). It is expected that conventional medicine would have been a great choice of disease treatment because it is scientifically proven remedy. But the increasing cases of antimicrobial drug resistance is becoming a serious global problem that is now promoting the search for new organic molecules with antimicrobial characteristics that serve as readily available raw materials for new drug synthesis. (Akaochere *et al.*, 2009). All African countries, of which Nigeria is inclusive, are endowed with an excessive amount of plants with reputable medicinal properties which are yet to be harnessed for new antibiotics synthesis.

Herbal medicines are also well patronized in developed and developing countries around the globe. World Health Organization (2002), said that in spite of the orthodox medicine introduction by the Europeans, as much as 80% of Africans still make use of herbal preparations as part of the primary health care (WHO, 2002).

Ginger usually produce flower whose rhizome, is generally used as a folk spice and medicine. Ginger is commonly called "Atale, ata-ile" in yoruba, "cithar" in Hausa and "jinja" in igbo. It can be used in many dishes and as ginger tea with honey. The juice and powder of ginger roots are often used as spice to flavour various dishes such as seafood, snacks, and non alcoholic beverages (McGee, 2004). The medicinal properties of ginger include: anti-inflammatory, antifungal, anti-vomiting, analgesic, anti-ulcer. It is also used to treat cold induced diseases such as asthma, cough, rheumatism, etc.

Ciprofloxacin is a broad spectrum antibiotic of the fluoroquinolones class. Many infection ssuch as bone and joint infections, diarrhea, typhoid fever, respiratory tract urinary tract infection, skin infections It can be taken orally, in eyedrops, or intravenously. It is used against both Gram-positive and Gram-negative bacteria infections.. It inhibits DNA gyrase, and a type 11 topoisomerase, topoisomerase IV, required to separate bacterial DNA, leading inhibition of cell division ((Drlica and Zhao, 1997).

Concurrent use of orthodox and herbal medicine is practiced in many rural and urban areas in Africa, including many communities and cities in Nigeria. Interactions may be taking place most likely, without being observed in people who are habitual use of orthodox medicine and herbal drugs. These interacttions could be: synergistic, antagonistic, indifferent, or addictive. The aim of this study was to evaluate the antibacterial interaction of crude methanol extract of *Zingiber officinale* with Ciprofloxacin, the therapeutic implications of the use of *Zingiber officinale* as a herbal remedy by patients who may be placed on ciprofloxacin for the eradication of one form of bacterial infection or the other.

Materials and methods

This study was carried out in the Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, Delta State University Abraka. Antimicrobial Agent: Ciprofloxacin (Shalina Laboratories, India).

Reagents: Methanol, disinfectant, methylated spirit, iodine, safranin dye, crystal violet, kovac's reagent, hydrogen peroxide, lugols iodine and absolute ethanol.

Media: Nutrient agar, nutrient broth, Mueller-Hinton agar, MacConkey agar and mannitol salt agar.

Microbial culture: *Staphylococcus aureus, Bacillus subtilis and Escherichia coli*

Glass wares and other materials: Sterile bottles (Bijou), sterile swab sticks, sterile petri dishes, test tubes, test tube racks, wire loop, spatula, blade, Bunsen burner, cork borer(6mm), sterile syringes (2ml and 5ml), white transparent bucket, muslin cloth, beakers, conical flask, measuring cylinder (100ml), aluminum foil, cotton wool, masking tape, disposable gloves, nose masks, sterile water, white laboratory coat, *Zingiber officinale* rhizome.

Instruments: Autoclave, incubator, weighing balance, hot air oven, refrigerator, microscope.

Collection of samples

Rhizomes of *Zingiber officinale* were purchased from Abraka main market, Abraka, Ethiope East Local Government Area of Delta State. The samples were then taken to the Pharmacognosy Department Abraka for authentication.

Methanolic extract of Zingiber Officinale preparation

The rhizomes were peeled, washed and then cut into small sizes. They were then sun dried for five consecutive days and then blended into fine powder. The fine powder (100g) was extracted using 600ml of methanol and 150ml of water (80:20) by the cold maceration method for 168 hours. The extract was filtered using a muslin cloth, and concentrated by evaporating the excess solvent using a hot air oven. The extract after concentration weighed (29.8 g), which gave a percentage yield of 29.8.

Preparation of plant extract for phytochemical screening

Two **(**2g) of the extract was dissolved in 40ml of a suitable solvent to produce a 5% weight per volume extract solution. Methanol was used as a suitable solvent, since methanol was used in extraction.

Phytochemical screening of ginger (Zingiber officinale)

The sample (ground ginger powder) was subjected to phytochemical tests for plant secondary metabolites such as, tannins, saponins, steroids, flavonoids, phenols, terpenoids, cardiac and glycosides alkaloids. (Trease and Evans method,) Sofowora, (2008).

Preparation of innoculum

Nutrient broth weighing 0.19g of dissolved in 15ml of distilled water, and transferred into a beaker. The solution in the beaker was poured into three bijou bottles. The three bijou bottles were sterilized in an autoclave of 121°C for 15 minutes and allowed to cool. Thereafter a flamed wire loop was used to inoculate the organisms (*Escherichia coli, Staphylococcus aureus, and Bacillus subtilis*) into the bijou bottles. The bijou bottles containing the organisms were incubated at a temperature of 37°C for 24hrs. Cheesbrough, M. (2006).

Serial dilution of extracts of Zingiber officinale

Seven test tubes previously sterilized were used for the serial dilution of the *Zingiber officinale* extract. Then 3.2g of the methanol extract was weighed into a test tube containing 4ml of sterile water and was shaken very well until the extract dissolved. Then to the rest six test tubes, 2ml of sterile water was measured into them using a sterile syringe. 2ml of the dissolved extract was then transferred from the first tube into another test tube containing 2ml of sterile water, this was shaken and 2ml transferred also from it into the next tube. This was done continuously until the last test tube contained 2ml of the solution, 2ml

was then discarded from it so that each test tube had 2ml of solution in it. The concentration of the extract in each of the tubes was 800, 400, 200, 100, 50, 25, 12.5mg/ml respectively.

Determination of zone of inhibition of extracts of Zingiber officinale

The sensitivity or preliminary of the extracts of *Zingiber officinale* and ciprofloxacin was determined employing agar well diffusion method. Six sterile petri dishes, labelled according to the different concentrations and organisms were placed on an already disinfected working table. 20ml of sterilized mueller-hinon agar was poured into each of the petri dish and allowed to solidify. Then sterile swab sticks were used to collect the organisms and swabbed on the respective petri dishes containing the organisms (*Escherichia coli, Staphylococcus aureus, and Bacillus subtilis*). A 6mm sterile cork borer was used to bore holes in the solidified agar on each petri dish. Using a 2ml sterile syringe, 0.5ml of each concentration of the extract was added to their respective holes in the petri dish. The test was repeated for all the organisms, and two replicate tests were performed. After 30 minutes, all the petri dishes were carefully packed with a masking tape. All the petri dishes were incubated for 24hrs at a temperature of 37°C. Control used against each test organism was methanol (negative control) and ciprofloxacin (positive control). Zones of inhibition was observed, measured and recorded after 24hrs incubation.

Determination of minimum inhibitory concentration (mic) of extracts of Zingiber officinale

This was done using agar dilution method. Serial dilutions of the extract were made with sterile water, by weighing 3.2g of the extract, to obtain concentrations between 800mg/ml and 12.5mg/ml. A volume of each concentration equal to 1ml was transferred into the labelled sterile petri dishes, and made up to 20ml with molten agar and then allowed to solidify. After solidifying, each plate was divided into three sections and labelled according to each microorganism. The surface of the agar was then streaked with the isolates using a wire loop. An overnight broth was used for this experiment. All the petri dishes were incubated for 24hrs at a temperature of 37°C. After 24hrs incubation, the MIC was taken to be the lowest concentration which showed no visible growth of each of the test isolate on the agar surface. The same procedure was repeated for ciprofloxacin. Cheesbrough, M. (2006).

The evaluation of interaction between Zingiber officinale extract and ciprofloxacin against Staphylococcus aureus, Bacillus subtilis and Escherichia coli

Mueller Hinton agar was prepared according to manufacturer's specification and autoclaved at 121°C for fifteen (15) minutes. The agar was allowed to cool before pouring 20ml into each petri dish and allowing it to solidify. Then serial dilutions of ciprofloxacin and *Zingiber officinale* extracts were made to get the MIC's for the various organisms. These dilutions were made for evaluation of their combined effect against *Staphylococcus aureus, Escherichia coli, and Bacillus subtilis*. To evaluate the interaction between *Zingiber officinale* and ciprofloxacin, six sterile petri dishes were used,since the procedure was done in duplicates, and labeled as ciprofloxacin, *Zingiber officinale* extract and *Zingiber officinale* extract plus ciprofloxacin and the organisms accordingly. The test organisms were then swabbed using sterile swab sticks on their respective plates, after which, using a 6mm cork borer, four (4) wells were punched into each of the plates respectively, and the exact concentrations of the ciprofloxacin and the Zingiber *officinale* extract obtained as their MIC was placed into the wells. In the wells labeled ciprofloxacin plus *Zingiber officinale* extract respectively, the exact concentration of the MIC gotten for each respective organism was placed into the wells. The plates were then incubated for 24hrs at 37°C and the zones of inhibition were observed. A meter rule was placed across the zones of inhibition, and measured from one edge of the zone to the other edge. Inhibition zone diameter (IZD) was reported in millimeters. The combined effect of the antimicrobials on the test organisms was determined and recorded

Cheesbrough, M. (2006).

Calculation of percentage yield of the extracts

Weight of powdered plant material = 100mg

%yield = Final weight/Initial weight × 100/1

Weight of methanol extract = 29.8g

% yield = 29.8/100× 100/1 = 29.8%

Results and discussion

The phytochemical composition of *Zingiber officinale* as shown in Table 1 showed the presence of alkaloids, steroids, terpenoids, flavonoids and saponins and the absence of phenols, tanins, and cardiac glycosides.Saponins detected in the methanol extract of *Zingiber officinale* have been found to be an antibacterial substance on cell wall of many organisms. (Harborne, 1992). Flavonoids have been observed to help in the wound healing and the treatment of disease of the skin owing to their capability to counteract the inflammation and wound acidity. Plants that contain alkaloids are usually used for malaria treatment, cough, and cold.

Table 1. Qualitative analysis results revealing phytochemicals present in the methanol extract of *Zingiber officinale.*

S/N	Phytochemical constituents	Methanol extract
1	Alkaloids	++
2	Phenols	-
3	Tannins	-
4	Cardiac glycosides	-
5	Steroids	++
6	Terpenoids	+++
7	Flavonoids	+++
8	Saponins	++

Key: + present ++ very present, +++ very, very present – absent.

The phytochemical constituent of the methanol extract as given in Table 1 can be compared to those obtained by Okiki *et al.,* (2015). The constituents occurred in varying concentrations with the exception of phenol, tanins and cardiac glycosides which were absent in the methanol extract. It is able to contribute as much as 30% of the essential oils contained in ginger rhizomes. This compound usually produces its distinct flavor in ginger. Gingerol has been known to be active in an animal model of rheumatoid arthritis (Funk *et al.,* 2009). Ginger compounds have been observed to be active against enterotoxigenic *Escherichia coli* heat-labile enterotoxin-induced diarrhea (Chen *et al.*, 2007).

Table 2. Antibacterial activity of the methanol extracts of *Zingiber officinale* on *Staphylococcus aureus, Escherichia coli and Bacillus subtilis***.**

Concentration of Extracts (mg/ml)	Average Inhibition Zone Diameters (mm)		
	S. aureus	E. coli	B. substilis
800	7.5	6.5	5
400	6	6	4.8
200	7	5	5
100	5.5	4.5	4
50	4.5	2	2
25	1	1	-
12.5	-	-	-
Ciprofloxacin (+Control)	35	39.5	38
Methanol (-Control	-	-	-

The result from the experiment were obtained by measuring the zones of inhibition on the bacteria (*Staphylococcus aureus, Escherichia coli, and Bacillus subtilis*) and minimum inhibitory concentration of the extracts of *Zingiber officinale* (methanol) as well as that of ciprofloxacin. Table 3 gives the zone of inhibition of the methanol extract of *Zingiber officinale* against *Staphylococcus aureus, Escherichia coli,* and *Bacillus subtilis*.

Table 3. Antibacterial Activity of the Methanol Extract of *Zingiber officinale on Staphylococcus aureus, Escherichia coli, Bacillus subtilis*.

Concentration of extracts (mg/ml)	Minimum Inhibitory Concentrations		
	S.aureus	*E. coli*	*B.substilis*
800	-	-	-
400	-	+	-
200	-	+	-
100	-	+	-
50	-	+	-
25	+	+	+
12.5	+	+	+

Key: + = Growth, = - No growth

The largest zone of inhibition for *S.aureus* (7.5mm) was observed at 800mg/ml while the least zone of inhibition (1mm) occurred at a concentration of 25mg/ml. For *B.subtilis*, the largest zone of inhibition (5mm) was observed at 800 and 200mg/ml while the least zone of inhibition (2mm) was observed at a concentration of 50mg/ml. For *E. coli,* the largest zone of inhibition (6.5mm) was observed at 800mg/ml, while the least zone of inhibition (1mm) occurred at a concentration of 25mg/ml. Ciprofloxacin which was the positive control, gave varying zones of inhibition for each microorganism. The zone of inhibition for *S.aureus* was 35mm, *B.subtilis* was 38mm, *E.coli* was 39.5mm.

Table 4. Minimum inhibitory concentrations of Ciprofloxacin against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis*.

Concentration of Ciprofloxacin (mg/ml)	Minimum Inhibitory Concentrations		
	S.aureus	*E. coli*	*B. substilis*
2	-	-	-
1	-	-	-
0.5	-	-	-
0.25	+	-	-
0.125	+	+	-

Key: + Growth, - No growth.

The negative control which was methanol, gave no zones of inhibition. Since the plant recorded high zones of inhibition against the three organisms, it means the plant can be used to treat infections and diseases caused by those organisms particularly if the active ingredients are being isolated. So, this can be used to treat infections caused by these organisms which is in line with claim of Peggy and Ody. It has been claimed that this plant material can be used in the treatment of variety of human ill-health conditions such coughs, migraines, struck amenorrhea, athlete's foot, viral infection bursitis, cold, flu, fever, kidney stones, chronic fatigue and Reynard's disease (Peggy, 2006).

Table 5. The evaluation of interaction between *Zingiber officinale* extract and ciprofloxacin against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.

Organism	Meth Extract (mg/ml)	Cipro (mg/ml)	Meth IZD (mm)	Cipro IZD (mm)	Meth and Cipro IZD (mm)	Mean IZD of Meth (mm)	Mean IZD of Cipro (mm)	Mean IZD of Cipro and Meth (mm)
S .aureus	50	0.5	7	16	27	7	15	26
			7	14	25			
E. coli	800	0.25	6	15.5, 20.5	24	6	18	23
			6		22			
B. substilis	50	0.125	14	20.5	28	11	9.3	26
			8	18	24			

Key: Meth-Methanol; Cipro- Ciprofloxacin; IZD- Inhibition zone diameter.

Table 3 gives the minimum inhibitory concentration of methanol extract of *Zingiber officinale* against *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis*. The MIC of the methanol extract against *Staphylococcus aureus* is 50mg/ml, *Bacillus subtilis* is 50mg/ml, *Escherichia coli* is 800mg/ml. It can be seen from the MIC results gotten that ciprofloxacin showed high activities against *E. coli, S.aureus and B.subtilis* as expected of it, since it is a broad spectrum antibiotic of the flouroquinolone class. Zingiber officinale rhizome extract also showed great activity against *S.aureus* and *B.subtilis*, except for *E.coli*, where it showed very little activity, inhibiting at just the highest concentration. The tested bacterial isolates were susceptible to methanol extract of ginger used. These findings match the earlier reports by Nascimento *et al.* (2000) and Peggy (2006). With the high susceptibility of bacterial isolates to ginger extracts gotten from this study, it is obvious that methanol extract of ginger can provide appropriate antimicrobial therapy for the treatment of some respiratory tract infections.

Table 4 gives the minimum inhibitory concentrations of ciprofloxacin against *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis*. The MIC of ciprofloxacin against *Staphylococcus aureus* is 0.5mg/ml, *Bacillus subtilis* is 0.125mg/ml, *Escherichia coli* is 0.25mg/ml.

The combined antibacterial effect of the methanol extracts of *Zingiber officinale* and Ciprofloxacin is presented in Table 4. Greater zones of inhibition (26mm) against *Staphylococcus aureus* was observed when the extracts and ciprofloxacin were used simultaneously compared to their individual sensitivity to *Staphylococcus aureus*. But this was not the case with *Escherichia coli* and *Bacillus subtilis* because less zones of inhibition were observed when the extract and ciprofloxacin were used simultaneously, compared to their individual sensitivity to these test organisms. It is clear that synergy was recorded for combinations of ciprofloxacin and *Zingiber officinale* against *Staphylococcus aureus*, while antagonism was recorded for the rest organisms. A probable explaination of this could be because both extract and antibiotic have different mechanisms of action or may be inhibiting two different steps in the same biosynthetic pathway of the organism, resulting in either synergism or antagonism at certain combinations. Ciprofloxacin known to act by preventing bacterial replication, through inhibition of DNA gyrase to while the mechanism of action of *Z.officinale* extract is yet to be completely clarified (Hallender *et al.,* 1982). It is important to note that two antimicrobial agents may interact antagonistically, if one is bacteriostatic and the other is bacteriocidal (Chambers and Saunders, 1996).

Conclusion

The study has provided a groundwork evidence of some kind of antibacterial interaction between the methanol extract of ginger and ciprofloxacin against *S. aureus*. There is an indication that combinations of ciprofloxacin and *Zingiber officinale* rhizome extract may have some usefulness in the chemotherapy of infections in which *S.aureus* is implicated.

Conversely, the combined effect of the interaction against *B.subtilis* and *E.coli* may not be so useful, since both organisms showed antagonism, and antibacterial effect can only be seen when each agent is used individually. The significant antibacterial effect observed supports the use of *Zingiber officinale* in traditional medicine for the treatment of bacterial infections and proves that herbal medicines can be as efficacious as Orthodox medicine in combating some pathogenic bacteria. In Nigeria where *Zingiber officinale* rhizome is commonly used and consumed socially and in traditional medicine, the therapeutic implications of consuming the rhizome alongside with ciprofloxacin cannot be ignored.

References

Kuhn MA. 2002. "Herbal remedies: drug-herb interactions 2002" Critical Care Nurse **22(2),** 22–34.

Osemene KP, Elujoba AA, Ilori MO. 2011. "A comparative assessment of herbal and orthodox medicines in Nigeria," Research Journal of Medical Sciences **5(5),** 280–285. http://dx.doi.org/10.4314/njnpm.v17i1.7

Chan K, Cheung L. 2000. Interactions between Chinese herbal medicinal products and orthodox drugs, CRC Press. http://dx.doi.org/10.4324/9780367805210/

De Smet PAGM. 1997. "The role of plant-derived drugs and herbal medicines in healthcare," Drugs **(54)6,** p 801–840. http://link.springer.com

Houghton PJ. 2009 "Synergy and polyvalence: paradigms to explain the activity of herbal World Health Organization. WHO traditional medicine strategy 2002-2005.

Opdyke DLJ. 1974. Food Cosmet. Toxicology. **12** (Suppl.) p 901. http://www.ncbi.nih.gov

O'Hara M, Keifer D, Farrel K, Kemper K. 1998. A review of 12 commonly used medicinal herbs. Archives Family Medicine **(7),** 523-536.

McGee H. 2004. On food and cooking. The science and lore of the kitchen.2nd Edition.Harold McGee(Ed). New York.p 425-426.

Harborne JB. 1992. Phytochemical Methods: A Guide to Modern Techniques of plant analysis (3rd ed) London*:* Chapman and Hall Publication.

Hallender HO, Dornbusch K, Gezelius L, Jacobson K, Karissan I. 1982 Synergism between aminoglycosides and cephalosporins with antipseudomonal activity: interaction index and killing curve method. Antimicrob Agents Chemother. **22,** 743-752.

Chambers HF, Saunde MA. 1996. Antimicrobial Chemotherapy. In: Hardman *et al* (eds). The Pharmacological Basis of Therapeutics, ed 10, New York, McGraw-Hill. P 1049-1073.

Riaz H, Begum A, Raza SA, Khan ZM, Yousaf H, Tariq A. 2015. Antimicrobial property and phytochemical study of ginger found in local area of Punjab, Pakistan International Current Pharmaceutical Journal **4(7),** 405-409. http://www.icpjonline.com/documents/vol4Issue7/03

Cheesbrough M. 2006. District laboratory practice in Tropical countries part 2 (2nd edition) Cambridge University press.

Sofowora A. 2008. Medical Plants and Traditional Medicinal in Africa, 3rd Edition, and spectrum books Ltd. Ibadan, Nigeria, p 23-25.

Okiki PA, Oyetunji O, Oso B. 2015. Antibacterial activity of Ginger (*Zingiberofficinale*) against isolated bacteria from the respiratory tract infections. Journal of Biology, Agriculture and Healthcare. ISSN 2224-3208 (Paper) ISSN 2225-093X (Online) **5,** 19.

Nascimento GGF, Locatelli J, Freitas PC, Silva GL. 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Brazilian Journal of Microbiology **31,** 247-256.

Chan K, Cheung L. 2000. Interactions between Chinese herbal medicinal products and orthodox drugs, CRC Press.

Chen Jaw-Chyun, Li-Jiau Huang, Shih-Lu WU, Sheng-Chu KUO, Tin-Yun HO, Chien-Yun Hsiang. 2007. "Ginger and Its Bioactive Component Inhibit Enterotoxigenic Escherichia coli Heat-Labile Enterotoxin-Induced Diarrhea in Mice". Journal of Agricultural and Food Chemistry **5(21),** 8390–7.