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Antimicrobial activity of ginger and onion extracts against enteric pathogens

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Abstract
The antimicrobial properties of extracts of *Allium cepa* and *Zingiber officinale* against different enteric pathogen were investigated by using agar well diffusion method. The result obtained revealed that, ethanolic extract of ginger gave the largest zone of inhibition against *S. aureus* and the lowest zone of inhibition against *E. coli* and *S. typhi* however the ethanolic extract of onion gave the largest zone of inhibition against *S. aureus* and the lowest zone of inhibition against *B. cereus*, *S. typhi*, *E. coli*. Methanolic extract of ginger gave the widest zone of inhibition against *S. aureus* and the lowest zone of inhibition against *E. coli* and the methanolic extract of onion gave the widest zone of inhibition against *S. aureus* and *B. cereus* and no zone of inhibition against *E. coli*. The result showed that ginger was more effective and produced remarkable inhibitory effect on the two out of four test organism when compared to the ethanolic and methanolic extracts of onion.

Keywords: Ginger, onion**,** antimicrobial activity, enteric pathogens

Introduction
The diseases which are born by food are gradually becoming a dangerous risk to public health. People become infected with microbes or intoxicated with enterotoxins produced from microbes. Proper control of microbes and effective sanitation will help to reduce the rate at which people become sick from eating the food. (Ortiz, 2015) [1]. Medicinal plants may be defined as any plant that can be put to culinary or medicinal use and include those we associate with orthodox drugs such as fox glove and opium poppy, as well as every day plants such as garlic. All the drugs from the past were substances with a particular therapeutic action extracted from plants. The use of herbs and medicinal plants as the first medicine is a universal phenomenon. (N Azu and Onyeagba, 2006) [2]. The increased usage of antibiotics has induced microrganism to acquire resistence factor which have become burning predicament (Abimbola *et al.*1993) [3] as a result there is an urgent need to find the alternative of chemotherapeutic drugs in disease treatment particularly those of plants origin which are easily available and have less side effects (Khulbe and Sati, 2009) [4]. Spices in general show both antimicrobial and antioxident characteristics, inhibiting the growth of bacteria and other pathogenic microbes. The antimicrobial activity of spices is due to specific phytochemicals or essential oils. The main factors that determine the antimicrobial activity are the type and composition of the spice, amount used, type of microorganism, composition of the food, ph value and temperature of the environment. Ginger is a medicinal plant that has been widely used all over the world, since antiquity, for a wide array of unrelated ailments inluding arthritis, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, fever and infectious disease (Ali *et al.*,2008) [5]. In human, ginger is thought to act directly on the gastrointestinal system to reduce nausea. Ginger has direct antimicrobial activity and thus can be used in treatment of bacterial infections (Tan and Vanitha 2004) [6]. "Generally recognized as Safe'' (GRAS) by the US FDA (ICMR bulletin, http //icmr.in /BUJUNE 03 new.pdf). Its antibacterial power is effective against preventing numerous intestinal problems that take place as a result of the alteration of the intestinal flora. This is ideal to avoid the formation of ulcers by eliminating the *Helicobacter pylori* a bacterium whose secretions of ammonia are responsible for many ulcers, specially those of the duodenum, and other gastric problem.it also neutralize the Gastric acid. The main antimicrobial agent is Gingerol a naturally occurring Phenol which disrupts the cell wall of bacteria causing cytoplasmic leakage. Ginger has the capacity to eliminate harmful bacteria such as *E. coli*, responsible for most of the diarrhoea, specially in children. *Bacillus cercus* which mainly cause Diarrohea, Nausea, ginger causes impact on the growth of this bacteria. It has been shown to reduce the stickiness of blood platelets, hence may help to reduce risk of artherosclerosis.

Onion (*Allium cepa*) is native of Israel. This has been cultivated from the ancient times by all the nations of Asia and Middle East. *Allium* is the largest and important representative genus of the Liliaceae family. Onions are easily propagated, transported and stored. Onions are effective against common cold, heart disease, diabetes, osteoporosis, cough and sore throat. It is rich in proteins, carbohydrates, sodium, potassium and phosphorus. Onion consist of its flower part and edible bulb part. Flowers are greenish white or purplish white, the bulbs are white.

The onion has both genetic and environmental components of relative pungency. Sulphur compounds in onion have also been shown to be anti-inflammatory both by inhibiting formation of Thromboxans and by inhibiting the action of platelet- a activating factor. Thiosulfinates condition antithrombotic benefit including antioxident activity, reduced serum cholestroland enhance platelet activity in vitro. For cardiovascular health its later effect is very important by reducing the probability that platelets aggregate in the blood, a major cause of heart attacks and stroks. Flavonoids are the chemical compound found in onion are active against microrganism and in vitro condition it has been shown its activity against wide array of microrganism. Tannins, allicin also possess antioxident and antimicrobial properties. The main antimicrobial agent in onion is Quercetin and allicin (thio-2- propene-1-sulfinic acid-5-allyl- esters), quercetin binds to the bacterial DNA gyrase while allicin inhibits certain thiol containing enzymes in the microorganism by the rapid reaction of Thiosulfinates. Several authors have reported pharmaceutical activity of extracts of Onion including anti-tumor anti-diabetic, anti-oxidant, anti-allergic. In vitro studies have shown Onion to possess antibacterial, antiparasitic, and antifungal activity.

Therefore keeping in view the significance of Ginger and onion the present work was carried out to study antimicrobial effects of these spices against food borne pathogenic bacteria.

Materials and Methods

- **Place of work-** The present study entitled Antimicrobial activity of Ginger and Onion against enteric pathogen was carried out in the Centre for Microbiology, Department of Botany, Ewing Christian College (ECC) – Allahabad, 211003.
- **Study sample**- Two plants i.e. Ginger (*Zingiber officinale)*, Onion (*Allium cepa)* used in this study were purchased from the local market of Gaughat near E.C.C. for determining the antimicrobial activity of the Ginger and Onion extracts. Four bacterial strains were used viz. *Escherichia coli, Staphylococcus aureus, Salmonella typhi and Bacillus cereus.* The used bacteria were purchased from the Department of Industrial Microbiology, Sam Higginbottom University of Agriculture Technology and Sciences, Mahewa, Allahabad- 211007 and were maintained in the Centre for Microbiology, ECC, Allahabad.
- **Inoculum preparation-**Nutrient broth was inoculated with freshly subcultured bacteria and incubated at 37 ^{0}C for at least 24 hours. Such prepared inoculum was used to swabbed on Muller Hinton Agar (MHA) using sterile cotton swab to make a lawn of bacteria.
- **Preparation of extract**- For the study Methanol and Ethanol extract were prepared from Onion and Ginger separately. The fresh 200 gm of Onion bulb and Ginger rhizomes were washed, peeled, sliced and dried in oven at 80^{0}C for 7 days. After drying, Onion and Ginger slices were grind to fine powder separately using electric blender. The amount of Ginger and Onion powder obtained after grinding was soaked in 100ml. of 95% Methanol and 100ml. of 95% ethanol for 24 hours and the pulp obtained was left in a sterile glass container to allow for air dry. After this the extract was stored below ambient temperature untill required. (Azu and Onyeagba, 2006) [6]

Antimicrobial assay using disc diffusion method –The antimicrobial assay of spices was performed by disc diffusion method. All the experiment were performed under sterile conditions. The Muller Hinton agar plates were inoculated separately with test bacterial strain and evenly spread on entire surface of each plate. The sterile borer (5 m. m./10m.m. diameter) were dipped aseptically in 70%alcohol for 1 minute and placed over Muller Hinton agar plates seeded with bacterial culture, by using micropippet the Ginger and Onion extract were poured in separate wells created by borer. The plates were left at ambient temperature for about half hour and then incubated at 37 ^{0}C for 24 hours and then observed zone of inhibition. The diameter of inhibition zone was measured in millimeters. (Azu and Onyeagba, 2006) [6].

Results and Discussion

The purpose of this project was to investigate the antimicrobial properties of two of such spices Onion and ginger. Ethanolic extract of ginger showed widest zone of inhibition against *S. aureus* (25.66mm) followed by *B. cereus* (24mm) then *E. coli* and *S. typhi* both the bacteria showed zone of inhibition 11mm. Ethanolic of onion showed widest zone of inhibition against *S. aureus* (21mm), followed by *E. coli* which showed the inhibition zone 18mm in diameter, then *S. typhi* (11mm.) and *B. cereus* (9.66mm.). According to occurrence of inhibition zone, ethanolic extracts of ginger was more sensitive to the *S. aureus* and then *B.cereus* in comparison to the *E. coli, S. typhi.* Methanolic extract of ginger showed widest zone of inhibition against *S. aureus* (5.33mm) followed by *B. cereus* (5mm), and then *S. typhi* (2.33mm), *E. coli* (1.66mm) and this result indicate that methanolic extract of ginger was also more effective for the *S. aureus* in comparison to the other test bacteria. Methanolic extract of onion showed same widest zone of inhibition against *S. aureus* and *B. cereus* (4.33mm) and then *S. typhi* (3mm) but the zone of inhibition in *E. coli* was nil. The inhibition zone obtained against *S. aureus, B. cereus, E. coli* and *S. typhi* was less sensitive for onion extract in comparison to the ginger extract. On comparisons, the ethanolic and methanolic extracts of both the plant material for inhibition zone against these four test organisms, ethanolic extract gave better result in compare to methanolic extract. Since *S. aureus* and *B. cereus* both the bacteria are gram- positive and the cell wall of gram positive bacteria made up of thick peptidoglycon layer(generally 20-80mm) showed wide zone of inhibition due to the absence of the bacterial outer membrane where as in case of *E. coli* and *S. typhi* both the bacteria are gram-negative and the cell wall of gram- negative bacteria made up of much thinner peptidoglycon layer (generally 2-3mm)and this layer sandwiched between an inner cell membrane and a bacterial outer membrane which make the cell more porous and hence gram negative bacteria showed less zone of inhibition against gram –positive bacteria.

Table 1: Comparative antimicrobial activity of Ethanolic extract of Onion and Ginger

Test pathogen	Zone of Inhibition in mm	
	Onion	Ginger
S. typhi	13.00	11.00
E. coli	18.00	11.00
S. aureus	21.00	25.66
B. cereus	9.66	24.00

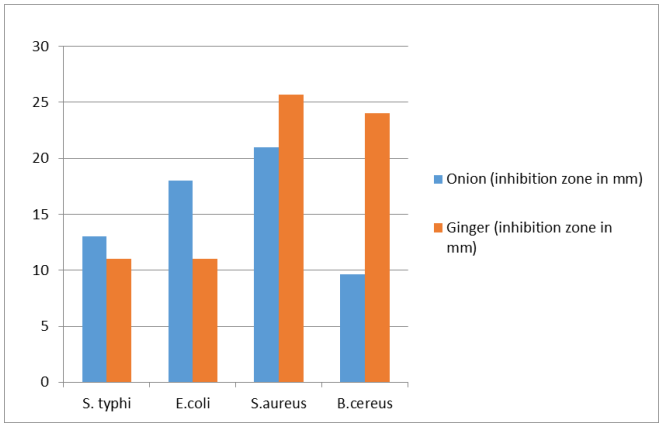

Fig 1: Antimicrobial activity of ethanolic Ginger and Onion extracts against enteric pathogens

Table 2: Comparative antimicrobial activity of Methanolic extract of Onion and Ginger

Test of Pathogen	Zone of Inhibition in mm	
	0nion	Ginger
S. typhi	3.00	2.3
E. coli	00.00	1.66
S. aureus	4.33	5.33
B. cereus	4.33	5.00

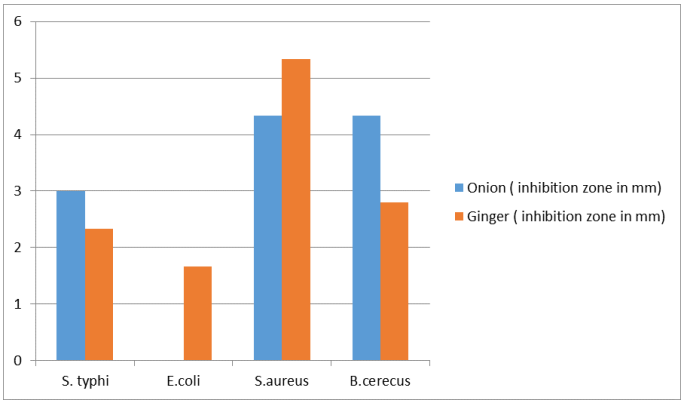

Fig 2: Antimicrobial activity of Methanolic extracts of Ginger and Onion against enteric pathogens

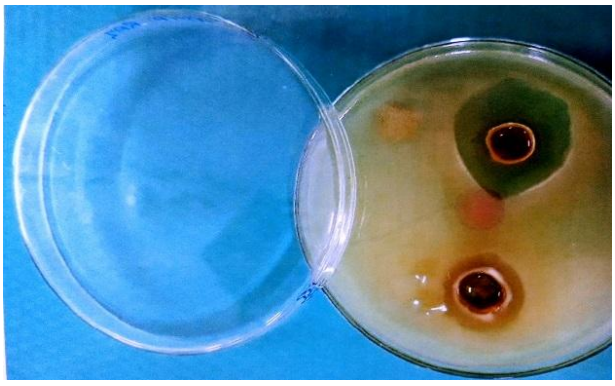

Plate 1: Antimicrobial activity of Ethanolic extracts of Onion and Ginger against *Bacillus cereus*

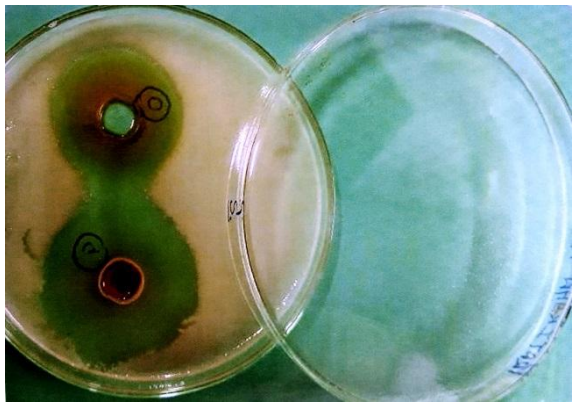

Plate 2: Antimicrobial activity of Ethanolic extract of Onion and Ginger against *Staphylococcus aureus*

Natural products including spices have been used for years because of their therapeutic and antimicrobial properties. The purpose of this project was to investigate the antimicrobial properties of two spices i.e. Onion and ginger. The result of this experiment showed *Staphylococcus aureus* to be more susceptible to the *Bacillus cereus, Salmonella typhi* and *Escherichia coli*. The decreasing susceptibility of tested pathogenic bacteria was observed in this order: *Staphylococcus aureus* >*Bacillus cereus* > *Salmonella typhi* > *Escherichia coli*. Both the gram negative and gram positive bacteria were sensitive to all tested extracts of onion and ginger but gram positive bacteria were more sensitive than gram negative bacteria. This result is in accordance with the findings of Iram Gull *et al*. (2012) [7]. Result were obtained by using agar well diffusion technique, based on this, methanolic extraction of Onion and Ginger showed little to no antimicrobial activity against either organism particularly *E. coli*, this was similar to the results gotten by Ortiz (2015) [1]. Kamrul Islam *et al.* (2014) [8] they determined the antimicrobial activity of *Zingiber officinale* (Ginger) in boiling soyabean oil extract. the result obtained by them showed the highest zone of inhibition against the *Salmonella spp.* and lowest zone of inhibition against *E. coli*, and ginger extract also showed the lower zone of inhibition against the *Staphylococcus aureus* compared to the gram negative bacteria. The result obtained by Kamrul Islam *et al.* (2014) [8] showed more activity against gram negative bacteria as compare to gram positive bacteria because they used Ginger for determining the antimicrobial activity in boiling soyabean oil extract but the result obtained in this study was more effective against gram positive bacteria as compare to gram negative bacteria because in this study the plant material used for determining the antimicrobial activity not in boiling ethanolic and methanolic solution but used at room temperature. It is also necessary to note that in this study the result obtained better in Ethanolic extraction in comparison to methanolic extract and this finding was somewhat similar to the result obtained by Onyeagba *et al.* (2004) [9] where they find the synergistic effect of ethanol extract of Ginger against gram positive bacteria *Bacillus spp, Staphylococcus aureus, Escherichia coli and Salmonella spp.* It is also important to note that experiment was affected by various factor such as concentration of spice extracts, volume of agar, and concentration of culture and incubation time. the stored spices extraction also affect (lower) the rate of inhibition as freshly prepared spices extract show better inhibition zone. The antimicrobial activity of onion be attributed to the presence of flavonoids and polyphenols which has been reported to have broad spectrum of antibacterial activity. The main

antimicrobial agent of Ginger is Gingerol a naturally occuring Phenol which disrupts the cell wall of bacteria causing cytoplasmic leakage. other chemical compound found in ginger are paradol, zingiberene, curcumin these compounds are found in fresh ginger juice, when ginger is cooked the story changes, then gingerols transformed into different compounds, which can alter both the flavour and pungency. Some of the advantages that herbal preparations have over the synthetic ones are that they do not act directly on the bacteria but create an adverse environment for them, thus threatening their survival and they have also been found to deter the development of resistent strains of microorganisms**.**

Natural products including spices have been used for years because of their therapeutic and antimicrobial properties. It is established in the study that spices reduce and inhibit the growth of food pathogens therefore the use of spices would decrease the chances of food poisoning and increase the food shelf life. several socioeconomic factors are major cause of miserable health condition of poor people includes; poverty, unhygenic conditions, overcrowding, contamination of food /water by poor sanitary practices, limited awareness of seriousness of food borne diseases, and importance of hyagiene. While living in such condition, use of such spices in diet can reduce the risk of food contamination, protect the consumer from the food borne diseases, improve their health status and combat with the foodborne disease by using small quantity of such spices in diet.

In conclusion, the results of present study have provided the justification for the therapeutic potential of spices. The pratices of using such spices as supplementary or olternative medicine in developing countries will not reduce only the clinical burden of drug resistence development but also the side effects and cost of the treatment with allopathic medicine. Further clinical evaluation of spices in *in* vivo experiment is required to be carried for low cost treatment with few side effects and for prevention of recurrent infection.

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