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Research Paper

''EFFECTS OF EXTRACTS OF TRADITIONAL INDIAN SPICES, GARLIC (ALLIUM SATIVUM) AND GINGER (ZINGIBER OFFICINALE) ON THE GROWTH RATE OF ESCHERICHIA COLI(E. COLI) AND BACILLUS SUBTILIS (B. SUBTILIS)."

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ABSTRACT **The present study was designed to estimate antimicrobial effects of the Garlic (Allium sativum)and Ginger (Zingiber Officinale) extract. The medicinal properties of garlic and**

xtract. The medicinal properties of garlic and ginger were very well recognized since ancient times *in India and have been used as an important constituent of Ayurvedic Medicines to cure many diseases and infections.Two potent pathogens, responsible for many infections, like food borne illness, Escherichia Coli (E.coli) and Bacillus Subtilis (B.Subtilis)were selected. The present investigation was carried out using the nutrient broth dilution method and the growths of both of these microorganisms were asessed against different volume of garlic and ginger extract respectively. Their growth was examined after 24-h by spectrophotometric analysis. The result showed that both garlic extract and ginger extract had good inhibitory action against microbial growth. Garlic extract proved to give satisfactory results as a potent inhibitory substance against both the microorganism while ginger extract showed medium activity.*

KEYWORDS :Garlic, ginger, antimicro- bial, E.coli, Bacillus sp.

INTRODUCTION

In the Indian Subcontinent, the use of dietary supplements, local kitchen spices and herbal remedies are popular for the people. They provide a short-term effect of medical conditions like inflammation, pain and swelling, while they have antimicrobial, anti-inflammatory and antimutagenic actions as their long term effect. There is in fact, a strong need of scientifically recognizing a strong relationship between beneficial properties of spices and their use in food on human health.Food borne pathogens play a major role in causing severe health hazards to population as they are widely spread in the environment. *E.coli* is one such opportunistic food borne pathogen often leading to infection causing severe diarrhea, occasional kidney failure and other severe health problems. Bacillus species, due to their capability to form heat stable toxins also constitute major food borne pathogen, leading to severe medical conditions. Different spices showed a vast range of inhibitory effect towards food spoilage and industrial yeast, out of which a few exhibited a complete inhibitory effect whereas others were insignificant to the action of spices against them (Sofia, et. al., 2007).In the present study spice pure extract from garlic and ginger were applied to study their effect on *Escherichia coli (E.coli)* and *Bacillus Subtilis (B.Subtilis)* microorganisms.*Escherichia coli (E.coli)* is a rod shaped, Gram-negative bacterium that is most commonly found in the gut of both humans and animals most of which are harmless.*E.coli* is one of the most commonly present organism in feces, so it is known as an indicator organism, and thus when food or water bodies are contaminated by fecal discharge it can directly cause its infection and can be fatal in many cases.*Bacillus Subtilis (B. Subtilis)* is a soil-dwelling, Grampositive, rodshaped bacterium. Food borne illness in humans causing severe nausea, vomiting and diarrhea has been shown by certain harmful strains of this bacterium. This condition arises due to survival of the bacterial endospores when food is inappropriately cooked. Under unfavorable conditions, bacterial vegetative cells form spore in order to survive. In a few patients symptoms, may last longer (Kotiranta A, et. al., 2007 & Roberts, T. A., et. al.,1996).*Allium sativum*, commonly known as garlic, is a species in the onion genus, and was proven to be a very effective antimicrobial agent when its extracts completely inhibited the growth of many Grampositive and Gramnegative organisms as well as some fungi. Garlic is a plant, which kills bacteria, fungus, parasites and lowers glycaemia and cholesterol and have liver protector property and includes antitumor agents. The antimicrobial activity of garlic is attributable to allicin inside it (Guo JJ *et al*., 2012; Fuji- sawa H *et al*., 2008). It is also declared that components including sulphur in garlic and also bioflavonoids like quercetin and cyanidin in it have great value in preventing diseases and infectionsalong with other anti microbial oils like clove oil (Nzeako, B. C., et. al., 2006.)Ginger (*Zingiber Officinale*), a member of the Zingiberaceae family, is a rhizomatous plant recognized as popular spice used in the daily diet in many Asian countries. In addition, it has been reported that the main ingredients of ginger like volatile oil, gingerol, shogaol and diarylheptanoids work as antioxidant, anti-inflammatory, anti-lipid, anti-diabetic, analgesic, antipyretic and antitumor (Hasan et. al., 2012). Zingerone may have activity against enterotoxin producer strains of *E.coli*which is already proven in mice (Chen, J.C., et. al., 2007).At present, there is a growing interest to detect natural compounds characteristics and activities, like plant extracts of herb and spices for the preservation of foods, flavor characteristic and sometimes show antioxidant activity as well as antimicrobial activity. This gives the stimulus for our present study to focus on garlic and ginger extracts.

Materials and method:

Two wellknown and commonly used spices known for their antimicrobial activities, namely garlic and ginger were verified against two opportunistic pathogens of significant importance, namely *Escheria coli(E.coli)*and *Bacillus Subtilis (B. Subtilis)* (Sofia, et . al., 2007, Guo JJ *et. al*., 2012 & Fujisawa H *et. al*., 2008).Garlic and ginger were procured from the local market. Both of them were first cleaned using tap water in order to remove any dirt or debris, and later using sterile distilled water. Samples were dried in a laminar flow biological safety cabinet. Five hundred grams of both garlic and ginger was skinned and homogenized using an aseptic blender and the extract was sieved using sterile muslin cloth. The extract was considered of 100 % concentration. Different volumes of garlic and ginger extract were directly used in experiments.The microorganisms used for antibacterial activity evaluated were obtained from the Department of Microbiology, B.E.T.S Science College, Palanpur, Gujarat, and 222

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they were Gram-positive bacteria *Bacillus Subtilis(B. Subtilis)* and Gramnegative bacteria *Escheria coli (E.coli).*

Methodology for Experimentation

15 mL nutrient broth was added in test tubes through a pipette and were sealed at the top with a cotton plug respectively. Sterilization was carried out in an autoclave at 121ºC and 15psi for 15 minutes.24 h old culture was was added in 11 test tubes and *B. Subtilis* culture in rest 11 test tubes respectively.Concentration of 0.4, 0.8, 1.2, 1.6 and 2.0 mLof garlic extract and ginger extractswere added in each test tube accordingly and were labeled.A total volume of 17.1 mLwas

made up by adding nutrient broth to it. The test tubes were inubated at the room temperature. The results were observed after 24 h of incubation by taking the optical density (OD) by spectrophotometric analysis.

Spectrophotometric Analysis

The present readings were taken on Systronic 161 spectrophotometer. Dark filter was set for air zero calibrations. Uninoculated nutrient broth was added in 2mL cuvette and OD was taken at 540 nm. The contents were removed and discarded properly. The cuvette was rinsed thoroughly with distilled water and cleaned with tissue paper. The procedure from step 3 and 4 were repeated for test samples of the respective *E.coli* and *B. Subtilis* in the respective test tubes having varying volumes add- ed int them respectively. The readings were taken accordingly.

Results and discussion:

Graph 1: Spectrophotometric analysis of garlic extract against different volumes of *E.coli* and *B. Subtilis.*

Graph 2: Spectrophotometric analysis of ginger extract against different volumes of *E.coli* and *B. Subtilis.*

Garlic and ginger are amongst the most commonly used spices in the Indian kitchen. Both of these spices have proven medicinal properties and most widely used as natural remedies in India. Garlic consist an active component called *allicin* which have anti-bacterial property. *E.coli* is the most common organism (normal flora) found in the small intestine of warmblooded animals. Some pathogenic strains of *E.coli* can cause severe gastroenteritis, urinary tract infections, and neonatal meningitis *Bacillus Subtilis* may cause disease related to food contamination.Optical Density (OD) was considered as the degree of growth in nutrient broth. As the no. of cells increases in the nutrient broth, its turbidity will also increase. Higher cell mass indicates the less inhibitory effect of the extract whereas lower cell mass shows the greater inhibitory effect. On the basis of BearLamberts law, it can be proved that higher OD represents the greater amount of cell mass whereas lower OD signifies the inhibitory effect of the extract.As shown in the above Graphs 1 and 2, extract concentration significantly affect the growth of both Grampositive and Gramnegative bacteria. Both the cultures, *E.coli* and *Bacillus Subtilis* were found to be sensitive for Garlic and Ginger extract. Positive control of the experiment was inoculated with *E.coli* and *Bacillus Subtilis* with no addition of any extract which means the growth of selected organisms without any inhibition. 0.999 And 0.890 OD were obtained in positive control of *E.coli* and *Bac illus Subtilis* respectively. Uninoculated nutrient broth was used as blank and its OD was 0.065.When 0.4 mL garlic extract was added in to the nutrient broth, OD for *E.coli* and *Bacillus Subtilis*were 0.368 and 0.253. The results indicated that, 63.17% and 74.68 % less OD was obtained in comparison to positive controls. As the garlic extract volume was increased to 0.8 mL, OD for *E.coli* and *Bacillus Subtilis* were 0.281 and 0.251 respectively. An interesting point was found about this result was that when garlic extract volume increase from 0.4 to 0.8 mL it did not show a significant decrease in cell mass. When the garlic extract volume was increased gradually in nutrient broth to 1.2, 1.6 and 2.0 mL, OD for *E.coli* were 0.179,0.081 and 0.011 respectively. In case of *Bacillus Subtilis,* OD was 0.201, 0.167 and 0.117 for 1.2, 1.6 and 2.0 mL garlic extract volume in the nutrient broth respectively. Here steady decrease in OD was observed for both the organisms as the garlic extract volume was increased. When 2.0 mL garlic extract was used 98.89% and 86.85 % less OD was achieved for *E.coli* and *Bacillus Subtilis* respectively.When experiment was done with ginger extract, inherent OD of ginger extract was observed. When 0.4 mL ginger extract was added in the nutrient broth, 0.504 and 0.631 OD was obtained for *E.coli* and *Bacillus Subtilis* respectively. As the extract volume is increased to 0.8 mL, the significant decrease in *E.coli* OD (0.133) whereas minor change was observed in *Bacillus Subtilis* i.e., 0.587. Ginger is reported as a weak antimicrobial agent with compare to garlic and here the same results were achieved (D Tagoe *et. al*., 2009). When 1.2 mL of ginger extract was added in to nutrient broth, a noteworthy decrease in OD i.e., 0.063 and 0.445 were attained for *E.coli* and *Bacillus Subtilis* respectively. While the addition of 1.6 and 2.0mL ginger in nutrient broth gave encouraging results and near about 100% inhibition was achieved in *E.coli*whereas only 31.92% inhibition was obtained for *Bacillus Subtilis.*In this study, a profound observation was made that both the antimicrobial agent were more affective on *E.coli* rather than *Bacillus Subtilis.* Physiology of *E.coli* shows that it is Gramnegative, facultative anaerobic and nonsporulating, whereas *Bacillus Subtilis* is rodshaped, endospore forming bacteria, and due to endospore it can tolerate extreme environmental conditions.

CONCLUSION

The present experiment was aimed for the observing antimicrobial activities of garlic and ginger extracts on E.coli and Bacillus Subtilis. It was found out that both garlic and ginger extracts had inhibitory effects on both the types of microorganisms taken into the study. Amongst the two spices used, garlic extract had a more significant effect on microbes than ginger extract; however, the effects of ginger extracts cannot be excluded.Usage of garlic and ginger extracts as a primary remedy against microbial infection or disease may prove beneficial in the first course. However, their significant inhibitory effect still needs to be studied further for proving them to cure or majorly affect the growth of studied microbes in due course. Further in depth studies are strongly recommended to certainly validate their dose and proportional effect in curing the disease caused by selected microbes.

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