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THE EVALUATION OF CINNAMON EXTRACT EFFECTS ON THE INTESTINAL *ESCHERICHIA COLI* AND ITS RECOMPENSE

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ABSTRACT:
Escherichia coli are a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium; it belongs to the genus Escherichia that is commonly found in the lower intestine of warm-blooded animals and this is an opportunistic microorganism which turns out to be pathogenic leading to multiple types of diseases such as urinary tract infections, traveler's diarrhea, bacteremia and cholecystis. In India 386,600 deaths are estimated annually by the infection caused by E.coli. The drugs which are in use for the treatment of E.coli are ampicillin, norfloxacin, gentamicin, cotrimoxazole, augmentin, ciprofloxacin, amoxicillin, streptomycin and nalidixic acid. But the E.coli is resistant to all of these drugs and has been converted to multiple drug resistant microorganism. As an alternative the Cinnamon extract is found to be promising one. The extraction of cinnamon through the process of aqueous extract and the solvent extraction according to the literature have proved that at the pH less than 2 and the temperature of 46^0-47^0 C can inhibit the growth of E.coli as it has the Cinnamaldehyde component, which acts primarily on the cell membrane by disrupting it. So, the incorporation of the cinnamon in the food can keep a check on the number of E.coli in the human intestine and can prevent from the diarrhea, urinary tract infections and other comorbidities.

Keywords: *Escherichia coli, drug resistivity, Cinnamon, agar well diffusion, filter paper discs, pH, temperature, protein synthesis inhibition.*

INTRODUCTION:

E.coli is a gram-negative, facultative anaerobic, rod-shaped, coliform bacterium which belongs to the genus Escherichia and is normal micro-flora of the human intestine. These are present in relatively low concentrations in the human intestine when compared to other microorganisms [34]. And these become opportunistic when the persons' immunity becomes low or when the microbial load increases due to the uptake of contaminated food or water, as reported by World Health Organization (WHO) [1]. These cause many diseases such as urinary tract infections, traveler's diarrhea, bacteremia and cholecystis along with ulcers in the intestine.

In India 386,600 deaths are reported annually by the infection caused by *E.coli* [35]. The children below five years of age groups are more susceptible to the *E.coli* infection among all the age groups [36]. The diaherria caused by the *E.coli* strain O157:H7 has no medications as the antibiotics can worsen the infection and can cause Hemolytic Uremic Syndrome [HUS] – mainly causes the kidney damage, which needs frequent hemodialysis. Many adverse effects of diaherria include the malnutrition. In India per year 3 lakh children die from diaherria, among which the more susceptible are from the age groups below 5. According to the report of Global Hunger Index (GHI) 2020, India was ranked 102 among 117 countries surveyed. This report analysis that, the malnutrition rate in India

makes the children more prone to the diaherria diseases. Children in the age group of 6-12 months are more susceptible due to their ill-developed immune system. Fatality rate increases due to this [38]. In case of the UTI, combinations of drugs are used along with nitrofurantoin. The UTI caused by E.coli are more in females than in males, due to anatomical variation, and the age groups more susceptible are 15-29 years of age in females and in males it is above 46 years of age group [37].

MATERIAL & METHODS:

1.1 The drugs used in treating the E.coli infection

The drug of choice for treating the E.coli related infections include ampicillin, norfloxacin, gentamicin, cotrimoxazole, ciprofloxacin, amoxicillin, streptomycin and nalidixic acid. But for treating the diaherria it is usually suggested to drink the electrolyte rich water or plenty of normal water in order to balance the electrolytes lost from the body.

1.1.1 Drug resistivity in E.coli

The drug resistivity in E.coli has increased over the years and the first line antibiotics such as ampicillin and gentamicin are of low use in recent scenario. The changing trends of E.coli when observed in the year 2005 were 70% for ampicillin and 85 for gentamicin. Where the multiple drug resistivity was 12% [39]. This is due to the excessive use of the antibiotics along with not completing the full course of the prescribed medications. This has led to the increase in the spectrum of beta-lactamase ring, which has led to the multiple drug resistance. The drugs prescribed for the UTI include nitrofurantoin and fluoroquinolone, as other drugs are not effective towards reducing E.coli infection due to the bacteria's resistivity [40].

1.1.2 Plant source as an alternative

In order to overcome this, multiple combinations of drugs are used to treat all kinds of E.coli infections [41]. Over the time the bacterium might be resistant to all these combinations. So the plant source medicines, which have the antimicrobial properties, can be used as an alternative to this problem [2, 42].

1.2 *Cinnamomum verum*

Cinnamomum verum (Cinnamon) is an effective spice found in the Indian history, which is extracted from the bark of the tree and due to its varied applicability it is used in the medicinal, cosmetic and food industry [2, 3]. Cinnamaldehyde (Figure1) [6] -a compound present extensively in Cinnamon- is an essential oil component and is an aromatic compound with a mono-substituted benzene ring with an aldehyde group [7]. It plays a role in reducing the blood pressure, fat deposited in the adipose tissue, reducing the glucose level and inhibiting the growth of *E.coli* in the human intestine [4, 5].

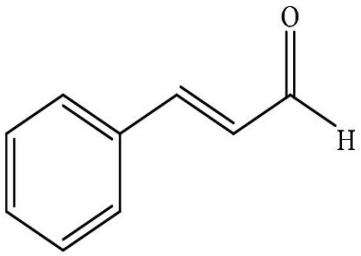

Figure 1: **Structure of Cinnamaldehyde [7]**

Some studies have proved that by reducing or inhibiting the in-vitro polymerization process and thereby degenerating the cells, cinnamaldehyde reduces/inhibits the cell division of E. coli in the human intestine [7, 8]. But the main component over here is the presence of Aldehyde group, which binds to the DNA proteins through the amines and in turn they interfere in the normal function of the bacterium. The study finds that, the action of cinnamon is different in different concentrations: when used in small concentrations, it inhibits the enzymes involved the process of cytokinesis. When used at sub-

lethal concentrations, it acts as an ATPase inhibitor. And when used in high concentrations the cinnamaldehyde acts on the cell membrane and disrupts it [43]. Not just the sub-lethal concentrations inhibit the ATPase concentrations, but, the amount of cinnamaldehyde present also contributes to the disruption (681-1362 µg/ml) [44]. Increasing the concentration of cinnamaldehyde increases the lethality of the bacterium (0.31 mg/g) [45].

As the cinnamon has these antimicrobial properties it is used by following some of the methods such as: the solvent extraction method- which mainly includes the extraction of cinnamon by adding ethanol [9]. The second one is aqueous extract where the water is added to the cinnamon [13, 14]. And the third method is the powdered form of cinnamon which is directly added to the microbe medium [6]. Along with these by varying the pH and temperature of the cinnamon, can produce the promising results.

2. IMPORTANT TECHNIQUES

2.1 Preparation of Inoculum:

- The sample was prepared by using the *Escherichia coli* (ATCC 25404™) inoculum and growing it in the Luria-Bertani (LB) broth at 37^{0}C for 24 hours.
- Required amount of sample was taken and spread on LB plates. This was left for drying for 15 minutes [46].

2.2 Solvent Extract Method of *Cinnamon*:

Solvent extraction is a process of separating two immiscible liquids, out of which one is water and the other is an organic solvent. This process was first developed for the analytical chemistry, so that all the metallic elements can be virtually separated. It is also known as Liquid –Liquid extraction. Steam distillation process is one of the types of solvent extraction, which is cheaper and more effective when compared to other methods [28]. This method is also considered as the safest method and the wastage of the products is limited over here.

2.2.1 Steam Distillation Process:

- 25.5g of cinnamon sticks were powdered and added to the round conical flask containing 100ml of water [47].
- To this solution 3-4 drops of Dichloromethane (DCM) was added and shaken vigorously to mix with the essential oil.
- Then the flask was tilted in order to separate the layer of dichloromethane and the distillate.
- After this the organic layer was transferred to a test-tube and this procedure of separation of DCM and collection of the organic layer was repeated three to four times.
- The test-tube containing the organic substance was kept in a boiling water bath until the DCM evaporates.
- Then the dark-yellowish solution of the cinnamon was obtained and was transferred into a micro-centrifuge tube and refrigerated. (Figure 3) [20]
- Required concentration of the extract was taken for further testing, that is the sensitivity test.

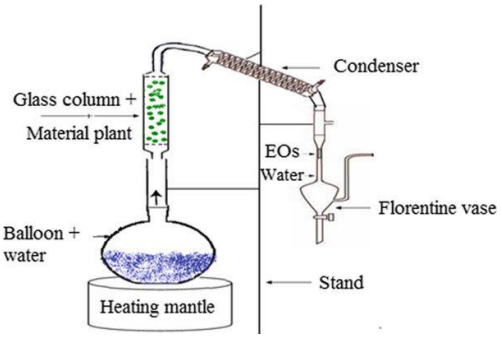

Figure 3: Steam distillation process [20]

2.2.2 Alcohol Dilutant Method:

- 70% ethyl alcohol was used as a dilutant in this paper [46].
- The series of dilutants of cinnamon essential oil (EO) was prepared in the order 0, 0.5, 1, 2 and 4%.
- Sterile paper discs were prepared and were placed in each dilutant and were left to dry.
- Each different sterile paper discs were placed in the respective petriplate containing the media along with E.coli, and were incubated at 37^{0}C for 24 hours for the observation of inhibition zone.

2.3 Sensitivity testing:

Sensitivity testing is the method which is used to test the sensitivity of the bacterium towards the antibiotic. It helps in the identification of the proper drugs and also helps in understanding the nature of the bacterium. It is usually determined by the size of the zone of inhibition. The zone of inhibition occurs when the antimicrobial substance is equal to or greater than the minimum inhibitory concentration, which acts upon the bacterium which has been inoculated on the medium [29].

2.3.1 Agar well diffusion method:

- The 40-micro liter of inoculums was swabbed in the solidified MHA medium and cooled for 15 minutes.
- 8mm diameter wells were created by using sterile stainless steel borer.
- The prepared cinnamon extract of varied concentration (105, 20%.....90%) were poured into the wells.
- This was incubated for 24 hours at 34^{0}C.
- After incubation the diameter of the zone of inhibition of various concentrations of extracts were measured.

2.3.2 Sensitivity testing using antibiotics/disc diffusion method:

- The antibiotic discs, such as, ampicillin and streptomycin were added in 10mcg concentration to the petriplates containing the inoculum and the medium.
- Then the petriplates were incubated in for 37^{0}C for 24hours.

2.4 Determination of Minimal Inhibitory Concentration (MIC):

Minimal inhibitory concentration is the concentration at which there will be no growth of microbes due to the activity of the antimicrobial agent after overnight incubation.

2.4.1 Broth dilution method:

- The MHA media was added to ten test tubes and the E.coli (40 micro liter) was added in the same concentration to all the test tubes.
- Then the serial diluted concentration of the cinnamon extract was added to the test tubes as 0.1, 0.2.....1 ml.
- Then the test tubes were kept for incubation overnight to get the required result.[21]

2.6 Varying the parameters of growth:

The parameters such as pH and temperature can play a major role in altering the growth of the microorganisms at the same time they may also inhibit the effect of cinnamon.

2.6.1 Temperature

- The cinnamon of varied concentrations had been kept at the high temperatures (usually in the pressure cooker as the food is usually cooked in that).
- After taking out from the pressure cooker, the cinnamon extract was added to the swabbed E.coli on the MHA medium.
- The petriplates were incubated for 24-48 hours and kept at 37^0 and observed for results.

2.6.2 pH

- The cinnamon extract was diluted to the high pH and the low pH value by adding

certain amount of acidic and basic solutions to the extract.

- Then the acidic solution was added to the swabbed E.coli MHA medium on one petriplate.
- In another petriplate the basic solution of the extract was added and in a third petriplate the neutral pH extract of cinnamon was added.
- Further all the plates were kept for incubation at the same temperature of 37^{0}C for 24-48 hours.
- Then observed for the results.[22,26]

ADJECTIVE STATEMENT

Here the experiments were performed in order to reduce the number of microbes, that is, *E.coli*, so as to get rid of the infection. Due to the excessive use allopathic medicines the bacteria began to develop resistance which is widely known as multiple drug resistance. So the researchers had performed the above mentioned study by using the Ayurvedic components and check their ability to inhibit the growth of microbes. The study also reveals that not just Cinnamon, but also wide number of spices in required concentration help in inhibiting the growth of microbes in their own way. The effect of Cinnamon on *E.coli* has wide applications along with inhibiting the microbial growth. These include reducing the blood sugar in diabetic patients and so on.

From the studies of Andrews JM (2001) [21] we will come to know the use of essential oil of cinnamon, which can reduce the growth of microbe. Thus essential oils are very useful and are not harmful as such. So these can be used in the preservation of food, especially the canned foods. Or else consuming the cinnamon water after having the fast foods can help to reduce the microbial load. But as per the studies done by Spisni E, et al., (2020) [18]the concentrations of the cinnamon added is also very important, as the increased load of it can harm the intestinal lining and may lead to some other infections. So it is prescribes to use around or more than 90% of cinnamon to reduce the intestinal E.coli and it is confirmed by the studies of Raeisi M, et al., (2015) [15]. Alteration of certain parameters required by the microbial growth can yield great results and further enhance its use. The varying level of pH can yield us the best results as proved and suggested by Chen YC, et al., (2003) [22].

The major parameters here are the temperature and pH. Altering them according to the concentration of the microbe present can lead very good results. Getting the promising results can lead to the accuracy of the experiment which can be implemented in further processes.

RESULTS & DISCUSSION

RESULTS

Antibiotic Sensitivity Test

The solvent extraction method is a promising one, in which, higher the concentration of the solvent of cinnamon added more will be the inhibition rate [47]. In the alcohol dilution method, the zone of inhibition was found more in the higher concentration, rather than in the lower concentration. Hence the concentration at 4% of the Cinnamon EO can be effective against the *E.coli* rather than at 2% of the concentration. In the concentrations 0 and 0.5% the zone of inhibition was with little difference. The zone of inhibition was 5.1mm, whereas in 4% concentration it was 7.1mm [46].

The zone of inhibition formed when the antibiotics such as ampicillin and streptomycin used was 2.3mm, which is less than the 2% concentration of Cinnamon EO [46]. This will

reveal that, the cinnamon is more effective against the gut E.coli rather than the synthetic antibiotics.

Minimal Inhibitory Concentration (MIC):

The MIC of the cinnamon on gut *E.coli* was found to be 4.88µg/ml and the inhibition diameter was 29.0mm. Whereas for ampicillin and streptomycin the MIC values were found to be 0.31µg/ml and 3.13µg/ml respectively. And the inhibition diameter was 20mm and 15.3mm [48].

Temperature and pH:

As the temperature and pH are the two main constituents for E.coli growth, differing these can alter the amount of *E.coli* present in the gut microflora. Higher the temperature and lower the pH the effect of cinnamon will be more on *E.coli*. As the outside factors sometimes contribute to the increase in the number of gut *E.coli*, it is necessary to use these two factors to prevent the former's growth [22, 26].

DISCUSSION:

The *Escherichia coli* can be destroyed by altering the pH of the medium, so that if the intracellular pH gets reduced then the destruction of *Escherichia coli* occurs [22]. The *Escherichia coli* can be killed or its activity can be halted/inhibited by increasing the concentrations of the Cinnamon oil used [15]. Higher the concentration of the oil used more will be the destruction. The medicinal herbs/plants including the cinnamon plant has the competence to reduce the harmful effect of *E.coli* on the intestinal colon. The traditional medicines and spices can be preferred in present era of allopathy [3].

The MIC of the synthetic antibiotics such as ampicillin and streptomycin was 20mm and 15.3mm respectively, but for the cinnamon it was 4.88µg/ml [48]. This indicates that the cinnamon is more effective against the gut *E.coli*. Whereas some results of the same paper suggest that by using the combination of the pre-used drugs such as ampicillin and streptomycin along with cinnamon oil can reduce the gut *E.coli* negative effect to some extent. This was preferred due to the multidrug resistivity of *E.coli* strains. The value of zone of inhibition varies according to the type of strain of *E.coli* present [33].

The stool samples collected in India showed that more resistant was towards ampicillin and 74% resistant was MDR in the *E.coli* isolated [30]. But when studied in the major countries, the data provides that, in these countries the gut *E.coli* strains were resistant to amoxicillin and ciprofloxacin [49].

CONCLUSION

Due to the MDR of the gut *E.coli* to almost all the drugs which were prevalent since many years, it is suggested to use the plant sources such as cinnamon as an antimicrobial drug. Along with these the combinations of the drugs can be used, but this may also lead to the MDR. So cinnamon is preferred due to its less/no side-effects on the human health.

Through the studies it was found that different concentrations of the cinnamon have different effects on the *E.coli* growth. If the concentrations of the cinnamon extract are increased to a greater extent the effect is more in terms of destruction of *E.coli*. To suppress *E.coli* in the human intestine, higher concentrations such as 90 %-100 % of cinnamon powder are required. As the concentration of the EO is increased, the zone of inhibition also increases. It is same for the temperature. But the acidic pH favors the reduction of *E.coli* in the intestine. Hence more the concentration of various cinnamon extracts more will be the effect.

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