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Screening for antimicrobial activity of *Coriandrum sativum* against potential enteric pathogens

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Abstract

The aim of this study was to understand the bioactivity and phytochemical study of methanol, ethanol and chloroform extracts of *Coriandrum sativum* (coriander) against bacteria including *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*. Coriander fruit is an annual herb originating from the Mediterranean countries. This herb is recommended in urethritis, urinary tract infection, and also for enteric diseases caused by bacteria. In the present study cold maceration technique was used to extract phytochemicals which are known to exhibit antimicrobial properties. The antimicrobial activity was tested using disk diffusion and macro broth dilution methods in order to determine the antibiotic sensitivity of the different extracts and the minimum inhibitory concentrations (MIC) of each extract against the pathogens. Moreover, minimum bactericidal concentration (MBC) was also determined. According to the test results, methanol extract of coriander showed significant antibacterial properties compared to the other two extracts. It was able to identify antibiotic properties against *Salmonella typhi* at higher concentrations (60mg/ml) of methanol extract but other two extracts were unable to show significant results against this bacterium. Moreover, methanol and ethanol extracts were able to show antibiotic properties against *Staphylococcus aureus* for all three concentrations (20mg/ml, 40mg/ml, and 60mg/ml). Therefore, it is clear that methanol showed significant results most precisely due to the presence of phytochemicals with higher antibacterial properties.

Keywords: *Coriandrum sativum*, *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, MIC, MBC

1. Introduction

Coriandrum sativum (coriander) is a member of the Apiaceae family and it's a widely used medicinal plant. South-East Asian countries are known to grow coriander as a culinary herb and the large-scale production of coriander is mainly exist in India, Southern Russia, the Ukraine and other East European countries[15]. Coriander mainly consists of fiber (23%–36 %,), carbohydrates (20%), fatty oil (16%–28%) and protein (11%–17%) [2]. The phytochemical screenings have shown that coriander contains essential oils, tannins, terpenoids, reducing sugars, alkaloids, phenoles, flavonoids etc. Phytochemicals are bioactive non-nutrient plant compounds which exhibit antimicrobial properties against pathogenic microorganisms. It was found that several components are responsible for the antimicrobial activity of herbs/spices[6]. Plant antimicrobials are phytochemicals which are generally divided into phenolic compounds (phenols), terpenoids, essential oils, lectins, alkaloids and polypeptide [20]. Mainly phenolic compounds have shown highest antimicrobial activity compared to other antimicrobial components such as alcohols, aldehydes, ketones, ethers and hydrocarbons. Research has found that this is due to the presence of hydroxyl (-OH) groups in phenolic compounds. Similarly, linalool (major component of EO), α-pinene, p-cymene, γ-terpinene, limonene and linalyl acetate present in coriander oil has also proved to be effective against several bacterial species[1]. Enteric pathogens are known to cause various diseases among people including enteric infections and diarrhoeal diseases [8]. Hence, it is important to

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understand the possible treatments to overcome these health problems. Microbial pathogens can produce toxins which can directly damage the host and can perform signal triggering host inflammation[8]. Microbial enteric pathogens, mostly rotaviruses, *Vibrio cholerae*, *Shigella spp*., *Salmonella spp* (eg: *Salmonella typhyrium*), enteropathogenic *Escherichia coli* (EPEC), and enteroaggregative *Escherichia coli* (EAEC) can induce acute diarrhea by ingesting contaminated food or water[16]. Moreover, enterotoxins produced by *Staphylococcus aureus* can result in food poisoning soon after its ingestion, [20] while *Escherichia coli* is responsible for producing toxins which induce diarrheal diseases.

In Sri Lanka typhoid fever is a major health concern compared to other enteric diseases, which is caused by *Salmonella enterica enterica typhi*. In Sri Lanka from 2000 to 2008, there was a decreasing trend of enteric fever cases, but in 2009 the notifications of typhoid are increasing. Increased numbers of infections are reported from the districts located in the dry zone (eg: Vavuniya, Mannar, Jaffna, Nuwara Eliya and Puttlam districts) due to lack of water in these areas. Therefore, the consumption of the contaminated water and food is abundant [5]. In Sri Lanka, to overcome these health problems studying of antimicrobial agents especially, natural compounds is important.

Nowadays the food industry uses preservatives such as benzoic and sorbic acids which are weak acids. These chemical preservatives are used to maintain the stability and safety of the food product on its whole shelf life. However, such components can result in microbial resistance against pathogenic bacterial species[10]. As substitutes natural products can be used because they are innately better tolerated in the human body and provide several advantages for the food industry [10]. Recently there has been a high demand for natural antimicrobial products as food preservatives and as medicines such as spices including *Coriandrum sativum*. These spices are known to contain significant antimicrobial and antifungal properties and have the ability to decrease the possibility of food poisoning and to increase the food safety and shelf-life of products [10].

Bacterial species such as *Staphylococcus aureus* and *Escherichia coli* are multidrug resistant and disinfectant bacterial types. The emergence of these species has increased rapidly, causing the increase of morbidity and mortality resulting in foodborne diseases [10]. Incidence of antibiotic resistance within bacterial species has increased since the commercial use of antibiotics became widespread. As examples, resistant to chloramphenicol, ampicillin, and trimethoprim by *Salmonella typhi* has resulted in many outbreaks in countries in the Indian subcontinent, Southeast Asia, and Africa [19]. Similarly *Staphylococcus aureus* has the ability to respond quickly to each new antibiotic with the development of resistance mechanisms [14]. Treatments for *Escherichia coli* infections have been increasingly challenging due to its resistance to most first-line antimicrobial agents [17]. From the present study, these microorganisms were tested for their antibiotic resistance against the natural antimicrobial compound; *Coriandrum sativum*, and the presence of phytochemicals were also analysed. Three extractions of *Coriandrum sativum* were obtained using the solvents; methanol, ethanol and chloroform. The extractions were carried out from *Coriandrum sativum* seeds using cold maceration technique. Antimicrobial activity of *Coriandrum sativum* was analysed against potential enteric pathogens including *Salmonella typhi, Escherichia coli* and *Staphylococcus aureus*, using the disk diffusion method and to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Coriandrum sativum* extracts, using broth dilution and spread plate methods, respectively. Since enteric diseases have become a common health issue to world, studying natural antibiotic compounds is important since such bacteria has become resistant to common antibiotics. Introducing new antibiotics may not provide a solution for the

antibiotic resistance but in presence of a disease alternative treatment options are important in case if the standard drug does not show any significant result against the pathogen. This study provides the idea of developing natural pharmacological compounds that are highly effective and less expensive. This can be obtained by extracting these synthetic compounds and introducing improved novel drugs to varieties of enteric infections.

2. Methodology

2.1 Extraction of *Coriandrum sativum.* The powdered sample of *Coriandrum sativum* (coriander) was obtained (10g) and extracted with 80% of methanol, ethanol and chloroform (40ml) using the cold maceration technique. For phytochemical tests, a 100mg/ml stock solution was prepared by mixing 1.0g of the extract with 10ml of 50% solvent.

2.2. Phytochemical screening of coriander extractions

2.2.1 Test for carbohydrates. 2ml of the extract was mixed with 2ml of Molisch's reagent. Then, a few drops of concentrated (conc.) H_2SO_4 were added along the test tube wall. The formation of a purple ring was observed as positive results.

2.2.2. Test for flavonoids. 1ml of the extract was added to a test tube. Then few drops of 10% NaOH were added to the tube. Yellow/red/brown colour formation was observed as positive results.

2.2.3. Test for tannins. 2ml of the extract was mixed with a few drops of 1% FeCl3 in a test tube. Green/blue/solid black colour formation was observed as positive results.

2.2.4. Test for saponins. 2ml of distilled water was mixed with 2ml of the extract. The tube was shaken vigorously. If saponins are present, formation of froth was observed as positive results.

2.2.5. Test for phlobatannins. 1ml of sample was mixed with few drops of 2% HCl. Formation of a red colour precipitate was observed as positive results.

2.2.6. Test for coumarin. 1ml of extract was mixed with few drops of 10% NaOH. Yellow colour formation was observed as positive results.

2.2.7. Test for phenols. 1ml of the extract was mixed with a few drops of 5% $FeCl_3$ in a test tube. Green colour formation was observed as positive results.

2.2.8. Test for oils. 1ml of sample was mixed with few drops of Sudan III. Pink droplets were observed as positive results.

2.2.9. Test for quinones. 1ml of the extract was mixed with 1ml of conc. H_2SO_4. Red colour formation was observed as positive results.

2.2.10. Test for terpenoids. This test was only done for methanol extract. 1ml of the sample was mixed with 2ml of 100% chloroform. Then 1.5ml of conc. H_2SO_4 was added to the mixture. The formation of a red colour ring was observed as positive results.

2.3. Testing the antimicrobial activity of *Coriandrum sativum*. As per the agar media, initially Nutrient, MacConkey and Muller hinton (MH) agar plates were made. Subcultures of *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* were prepared using streak plate method. MacConkey agar was used for both *Salmonella typhi* and *Escherichia coli* whereas nutrient agar was used for *Staphylococcus aureus*.

Disk diffusion was carried out for three extracts of coriander for the three microorganisms. 10ml of saline solution was inoculated with each microorganism using a sterilized loop. The turbidity was compared with the 0.5 McFarland solution using a Wickerham card. After saline solution reached 0.5 McFarland standard spread plating was done using MH agar.

Initially, 200µl of the saline suspension was added to the labelled agar plate and spread evenly on the agar. Then the stock solutions of the coriander extracts were diluted into 20mg/ml, 40mg/ml and 60mg/ml concentrations. First, gentamycin disks were placed on the agar plate followed by the filter paper disks which were dipped in negative control, 20mg/ml, 40mg/ml and 60mg/ml concentrated solutions for one minute. The zone of inhibition was measured after incubation.

Finally, the extractions which gave zone of inhibitions for each bacteria at all three concentration (20mg/ml, 40mg/ml and 60mg/ml) were subjected to broth dilution. Therefore, methanol and ethanol extractions of *Coriandrum sativum* were used to determine MIC against *Staphylococcus aureus.* After determining the MIC value, diluents which showed a clear appearance without turbidity were subjected to MBC.

3. Results and Discussion

Following table indicates the presence of phytochemicals in each extract of *Coriandrum sativum* (table 1)

Table 1. The presence of phytochemicals

Phytochemical	M	E	Chl
Carbohydrates	(+)	(+)	(+)
Saponins	(+)	(-)	(-)
Tannins	(+)	(-)	(+)
Flavonoids	(+)	(+)	(-)
Phlobatannins	(-)	(-)	(-)
Coumarin	(+)	(+)	(-)
Phenols	(+)	(+)	(+)
Oils	(+)	(+)	(+)
Quinones	(+)	(+)	(+)

Methanol extract (M), ethanol extract (E), chloroform extract (Chl), positive results (+), negative results (-).

80% methanol, ethanol and chloroform can extract a higher amount of phenols by creating a moderately polar medium. Due to impurities, water was not used as a single solvent because this interferes with phenol identification [13]. The highest extraction yield was obtained for chloroform compared to other extracts. It is strongly believed that higher molecular weighted solvents with low polarity, enables the easy extraction of substances with the same molecular weight solutes such as condensed tannins [13]. Increased molecular weighed components increase the sample yield. Therefore, higher molecular weight of the chloroform which also has a least polarity have contributed to highest extraction yield compared to others. Antimicrobial properties of coriander depend on different phytochemicals. Major antibacterial phytochemicals like phenols and oils were found to be positive in all three samples, which indicated higher antimicrobial properties. Compared to all three extracts methanol was able to give positive results for most of the phytochemicals showing that the most polar solvent with the least molecular weight extracts more phytochemicals. Chloroform showed negative results for flavonoids since it contains a hydroxyl group which can form hydrogen bonds with polar solvents like methanol and ethanol [7].

The presence of many phytochemicals in methanol compared to ethanol is due to its higher dielectric constant [12], or the higher water concentration in the ethanol extract due to less evaporation. This results in less concentration of phytochemicals which will not give visible results. During the Sudan III test, compared to methanol and ethanol extracts, chloroform extract gave significant results for oils. Since oils are hydrophobic and non-polar substances, as a non-polar solvent chloroform can effectively extract oils. Oils can be slightly soluble in polar solvents like methanol and ethanol due to attached polar molecules [3].

Disk diffusion results revealed zone of inhibition (7.1mm) for *Salmonella typhi* at only 60mg/ml concentration of methanol extract (Figure 1). For *Staphylococcus aureus* all three concentrations of methanol extract gave zones of inhibition;

20mg/ml (11.66mm), 40mg/ml (22.33mm) and 60mg/ml (23.33mm) (Figure 2). For chloroform extract of no inhibition zones were observed for all three microorganisms.

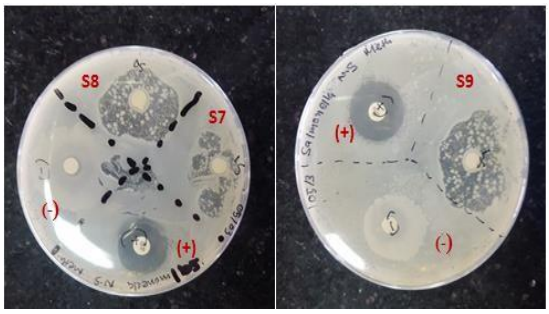

Figure 1: Disk diffusion results for Methanol extract against *Salmonella typhi* at 60mg/ml (S7, S8 and S9) concentration

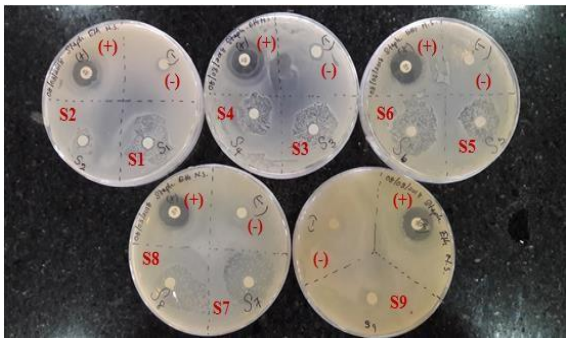

Figure 2: Disk diffusion results for Methanol extract against *Staphylococcus aureus* at 20mg/ml,40mg/ml and 60mg/ml concentrations

According to results, all three extractions did not give inhibitory zones for *Escherichia coli*, probably because as a gram-negative bacterium it has higher tolerance toward antibiotics. This even agrees with the inhibition of *Salmonella typhi* only at high concentration of methanol extract, which is also a gram-negative bacterium. The outer membranes of bacteria promote antimicrobial resistance and have the ability to interpret signals from antibiotics. Hydrophilic antibiotics (methanol/ethanol extracts) cannot cross the outer membranes of gram-negative bacteria. These bacteria show resistant to commonly used antibiotics, therefore only a few antibiotics are used to target these bacteria [11].

Excluding the chloroform extract, methanol and ethanol extracts exhibited zones of inhibition against *Staphylococcus aureus* (gram positive bacteria). Hydrophilic antibiotics use porin channels to enter into gram positive bacteria because they cannot diffuse across hydrophobic layer [9]. Similar mechanisms may have been used by methanol and ethanol extracts against *Staphylococcus aureus*. The results showed higher inhibitory zones for all three concentrations for ethanol extract, compared to methanol extract. This could be due to the presence of an antimicrobial agent present only in the ethanol extract which can significantly inhibit the growth of *Staphylococcus aureus*, or the agar medium and environmental factors have affected the bacterial growth. Compared to the positive control (gentamycin), 60mg/ml concentration of methanol extract has shown larger inhibition zone whereas ethanol extraction showed larger inhibition zones for all three concentrations. This indicates that both of the extracts show higher antimicrobial activity against *Staphylococcus aureus*.

Efficacy of the disk diffusion method always depends on the MIC value because it does not give a definitive value for the antimicrobial activity of the agent. In order to obtain the MIC results of methanol and ethanol extractions for *Staphylococcus aureus*, colony forming units (CFU) were initially determined as 1.18 x 10^6CFU/ml. Then broth dilutions were carried out where no turbidity was observed at 8mg/ml, 16mg/ml and 32mg/ml concentrations. Hence, minimum concentration that showed no turbidity was observed at 8mg/ml concentration for both methanol and ethanol extracts, the MIC value (x) was assumed to be at ($8 \geq x > 4$) mg/ml concentration. Therefore, the lowest concentration of the extract that inhibits the

growth of the bacterium is in between ($8 \geq x > 4$) mg/ml concentrations [18].

Since broth dilution results were observed for both methanol and ethanol extractions at 8mg/ml, 16mg/ml and 32mg/ml concentrated samples, they were subjected to determine the MBC value. For both extracts, MBC value was determined to be present at ($8 > x > 4$) mg/ml concentration. The determination of MBC is used to estimate bactericidal activity [4]. According to the MBC results for both of the extracts, the value should be present at ($8 > x > 4$) mg/ml concentration. This will be the concentration of the extract that kills >99.9% of the bacteria [18]. Since this kills the bacteria other than inhibiting the growth at MIC, this is the least concentration that gives highest antibacterial activity for a particular antibacterial agent.

Conclusion

According to the study, methanol and ethanol extracts can act as potential antibiotics against *Staphylococcus aureus*. Study shows that there is considerable antibacterial activity of methanol extract against *Staphylococcus aureus*, compared to other two extracts. According to the test results, methanol extract of coriander showed significant antibacterial properties compared to other two extracts. Also, it was found that antibiotic properties against *Salmonella typhi* at higher concentrations (60mg/ml) of methanol extract were effective against this bacterium. Based on the findings, this study may contribute future researches to develop new methodologies where medicinal plants like *Coriandrum sativum* to be used in alternative treatment methods to treat infections caused by common enteric pathogens.

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