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QUALITATIVE PHYTOCHEMICAL ANALYSIS AND IN-VITRO ANTIBACTERIAL SCREENING OF *Coriandrum sativum* and *Cinnamomum verum* AGAINST DIARRHEA CAUSING BACTERIA

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ABSTRACT

The antibacterial action of the two most popular spices in India *Cinnamomum verum and Coriandrum sativum* is an admirable research work since now. The purpose of this research is to utilize these samples against diarrhea-causing bacteria to slow down or inhibit their toxic effect on the body. Diarrhea is a very common and serious infection caused due to the ingestion of pathogenic microbes after unhygienic consumption of food products, if not treated properly then it can extend to a very harmful stage. The common bacteria responsible for diarrhea are *Escherichia coli, Salmonella, Campylobacter sp., and Shigella sp.* phytochemical analysis has been done to confirm the presence of antibacterial compounds like saponin, tannins, flavonoids, coumarins, and terpenoids in the sample which also will give health benefits to the body. Isolation and Identification of the bacteria were done to suspect diarrhea-causing bacteria. *Shigella and Escherichia coli* bacteria were identified after morphological and biochemical tests. Antibacterial screening of samples was done by the disc diffusion method to confirm their inhibitory action against the identified diarrheal bacteria. *Cinnamomum verum* has shown 13mm *and Coriandrum sativum* has shown 14mm zone of inhibition when tested against *Shigella* whereas *Cinnamomum verum has* showed 9.5mm *and Coriandrum sativum* has shown 10mm zone of inhibition when tested against *E. coli*. After knowing their ability to inhibit the bacterial action we can confirm that the spices extract is showing an

average but positive result and hence can be utilized further individually or in combined form for treating diarrheal infection.

KEYWORDS: Antibacterial, *Cinnamomum verum, Coriandrum sativum,* Diarrhea, Phytochemical analysis, *Shigella, Escherichia coli,* Antibacterial Screening.

INTRODUCTION

The human food chain primarily includes 'plants', 'animals', and products derived from them, including this, 'microbes' too plays a vital role in the chain and are naturally present in the soil, water, air, and also on the exterior surfaces of almost everything around us. Microbes are usually referred to as minute, single-celled organisms that are invisible to naked eyes. They can also be called microorganisms or microscopic organisms because their visibility is only under the microscope. Microbes are characterized in different sizes and have different life forms, majorly include Bacteria, Fungi, Viruses, Protists, and Archaea. (Fuerst, 2014). There are strong shreds of evidence that microbes are responsible for many chronic diseases like in a form of cancer and coronary heart disease. Any microorganism that has the potential to cause diseases is known as a pathogenic microorganism and further, if they multiply in an individual body it causes infection. (Mathew, 2014). The most common disease caused due to pathogenic organisms is 'Foodborne disease' which refers to any disease either poisonous or transferrable in nature, caused by means that enter the body through consumption of food. It usually occurs when people consume unhealthy food or food and drinks which are contaminated with pathogens or toxins. Some commonly known organisms which cause foodborne diseases include *Staphylococcus aureus, E. coli, Salmonella typhi and paratyphi, Listeria monocytogenes, Shigella spp., Clostridium botulinum, Campylobacter jejuni, Clostridium perfringens, Vibro cholera, Vibro parahaemolyticus. E. coli* is further divided into 2 types, Shiga toxin i.e. *E. coli* O157:H7, and non-Shiga toxin which includes enteropathogenic, enteroinvasive, enteroaggregative. (Vemula et al., 2012; Switaj et al., 2015).

The common symptom caused by these bacteria's is 'Diarrhea' and it is among one of the most common health complaints and can occur from mild, temporary condition to a life-threatening condition, possible causes due to many reasons like infection by bacteria, eating unhygienic foods which make stomach upset, infections by any other organisms and toxins, too many medications, malabsorption of food (poor absorption), intolerances to certain foods, etc. The most common pathogens responsible for diarrhea are *Salmonella, Bacillus cereus,*

Campylobacter, *Clostridium perfringens*, *Shigella*, *Campylobacter jejuni*, *Staphylococcus aureus*, and *E. coli*. (MacGill, 2020; Schiller, 2000).

Diarrheal illness is the second foremost cause of death; around 5.5 lakh people suffer and die because of diarrhea illness every year. It mostly lasts for few days but responsible for the loss of fluids and salts in the body that is basic for a healthy body. The different body reacts differently to bacterial infections, those who have a good immune system can easily tackle the situation with minor problems but those who have a weak immune system or those who are undernourished are mostly at a high risk of a life-threatening situation. (WHO, 2017). Diarrhea is a very serious issue around the world across all age groups; it causes children with an age group of 0-59 months and is responsible for around 2 million deaths every year. As per the global estimation near about 2 billion cases of diarrhea occur every year and also around 1.9 million children in developing countries under the age of 5 years die because of diarrhea, which tells that diarrhea is a very serious digestive disorder that needs precautions. (MacGill, 2020). The mortality rates in adults and children above the age of 5 are somehow lower as compared to under the age of 5, but still, the number is not very low, approximately 3 billion diarrhea patients are recorded annually. Diarrheal disease if not treated properly, patients have a high chance of causing disability due to this. Alone diarrhea kills around 2500 children daily which is more than the combined rate of diseases like AIDS, malaria, and measles. Every 1 out of 9 children around the world is suffering from diarrheal disease and deaths due to this. (CDC, 2015)There are some foods that worse diarrhea and should be avoided by the diarrhea patients like caffeinated drinks, foods rich in sweetness, honey, dates, nuts, soft drinks, lactose in dairy products, and anything which contains artificial sweeteners, etc. To prevent diarrhea one should maintain hygienic conditions like drinking safe water, eating safe & hygienic foods, washing hands properly before and after having food and after using washrooms, etc., promoting these kinds of sanitation habits may reduce the diarrhea rates by about one-third as the growth of diarrhea-causing pathogens will decrease. (MacGill, 2020).

Spices are a common member of every household and have the ability to give tremendous health benefits and have antimicrobial properties as well. Out of the most active spices, Coriander (*Coriandrum sativum*) belongs to carrot ancestors (*Umbelliferae*) and genus *Coriandrum*, is a well-known herbal and highly aromatic plant widely used as a spice in every household also called as a storehouse for bioactive compounds, its seeds have many

medicinal and healing properties like anti-inflammatory, anti-diabetic, anti-microbial, anti-oxidant, anti-septic, anti-hypertensive, and also have both nutritional and therapeutic properties. (Singh, et al, 2015), Ability to cure gastrointestinal complaints like griping pain, vomiting, anorexia, sub-acid gastritis, and diarrhea, it also has digestive stimulation and antibilious effect and it also improves glucose intolerance. *Coriandrum sativum* is considered an important spice and used for many purposes like as a flavoring agent, in the treatment of skin inflammation, high cholesterol level, diarrhea, menstrual disorders, mouth ulcers, blood sugar control, joint pains, toothaches, digestion problems, also use to treat bacterial and fungal infections, prevent food poisoning and many more. (Nadeem et al.,2013). The second most common and antimicrobial spice; Cinnamon (*Cinnamomum Verum*) belonging to *the Lauraceae* family, is a very essential and flavor-filled spice across the world. Its bark is also very popular and used not only for cooking but also as a medicinal source. Mainly pronounced as 'Dalchini' all over the country, is widely used in industries because of its strong aroma and essence and incorporated in varieties of perfumes, food products, and medicinal products. it also has many health benefits as it prevents bleeding, increases blood circulation in the uterus, lowers cholesterol levels, and has activities like anti-microbial, anti-inflammatory, anti-cancer, anti-diabetic, anti-oxidant, anti-fungal, antimycotic, etc. (Rao and Gan, 2014).

Coriander is a spice that is strongly effective against gram-negative bacteria like *E. coli, Pseudomonas aeruginosa. Enterobacter aerogens, Salmonella* typhi, and gram-positive bacteria like *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus*, and its seeds are helpful to cure indigestion. Spice is well known for its essential oil production which has antimicrobial properties. Coriander seeds can fight against some major bacteria include *Staphylococcus aureus, E. coli, Shigella sp., Salmonella typhi,* which are also responsible for causing diarrhea. (Chahal et al., 2017) Phytochemicals mainly present in coriander seed extract are Terpenoids, Steroids, Phenols, Flavonoids, Quinone, Coumarin these all show inhibitory action against bacteria as well as fungi in all the extracts. Antimicrobial activity of coriander oil which concluded that its leaves and seeds are effective against foodborne pathogens when tested *in vitro,* using its essential oil will increases the shelf life of food products along with providing health benefits. *Cinnamomum Verum* (Cinnamon) is also a spice that has antimicrobial activity against some common foodborne pathogens such as *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Salmonella* reported in a study. Phytochemicals mainly present in cinnamon bark are

alkaloids, saponin, flavonoids, glycosides, tannins, these all phytochemicals help inactivate the bacterial action in the body. A study about the activity of cinnamon extract has concluded that its extract Conc. can treat pathogenic bacteria. (Shan et al., 2007).

MATERIALS AND METHODS

Sample collection- The below sample's in Figure 1 i.e. *Cinnamomum Verum* bark and *Coriandrum sativum* seeds was collected from nearby local market and converted into fine powder form with the help of a mechanical grinder.

Figure 1: Collected Samples.

Method of extraction- Methanol extraction of samples was done by weighing 2gm each of the powdered samples in 10mL of methanol in a test tube. Mixed vigorously and kept undisturbed for 24 hrs. The supernatant of extracted samples was collected in the 5mL vials and was then preserved in the refrigerator throughout the experiment.

Qualitative phytochemical analysis

1. Test for Saponin (Foam Test)

2mL plant extract was diluted with 8mL of distilled water and shaken in a graduated cylinder for 15 minutes approximately; the appearance of foam layer of about 1 cm in plant sample indicates the presence of Saponin.

2. Test for Tannin (Ferric Chloride Test)

1 mL plant extract taken with 2mL of 5% ferric chloride ($FeCl_3$), the transformation of plant sample to dark blue or greenish-black color indicates the presence of tannin.

3. Test for Glycosides

1mL plant extract taken with 1mL chloroform ($CHCl_3$) and 1mL 10% ammonia (NH_3) solution, the transformation of plant sample to pink color indicates the presence of glycosides.

4. Test for Phenols

1mL of plant extract taken with 500µL ferric chloride ($FeCl_3$), the transformation of plant sample to blue or green color indicates the presence of phenols.

5. Test for Steroids

1mL plant extract taken with 1mL of chloroform ($CHCl_3$) and 1mL of concentrated sulphuric acid (H_2SO_4). The making of a brown ring at the meniscus in the plant sample indicates the presence of steroids.

6. Test for Flavonoids (NaOH Test)

500µL plant extract taken with 500µL of 2N sodium hydroxide (NaOH), with the yellow color presence add 200µL of concentrated hydrochloric acid (HCl) was added, further disappearance of yellow color in plant sample indicates the presence of flavonoids.

7. Test for Quinones

500µL plant extract taken with 250µL concentrated sulfuric acid (H_2SO_4), the transformation of plant sample to red color indicates the presence of Quinones.

8. Test for Anthraquinones

500µL of plant extract taken with 750µL 10% ammonia (NH_3) solution. The appearance of pink color precipitates in the plant sample indicates the presence of Anthraquinones.

9. Test for Terpenoids (Salkowski Test)

0.5mL plant extract taken with 2mL chloroform ($CHCl_3$) and 1mL concentrated sulfuric acid (H_2SO_4), the transformation of plant sample to red-brown color at interface indicates the presence of Terpenoids.

10. Test for Anthocyanin

1mL of plant extract taken with 1mL 2N Sodium hydroxide (NaOH), heating for 5min at 100ºC, conversion of plant sample to bluish-green color indicates the presence of anthocyanin.

11. Test for Alkaloids

1mLof plant extract taken with 75µL concentrated hydrochloric acid (HCl) following with 400µL of dragendorff's reagent. The appearance of orange-brown precipitate in the plant sample indicates the presence of Alkaloids. Figure 14 is represented as an observation for the following test.

12. Test for Phlobatannins

500µL plant extract and 500µL distilled water mixed with 100µL concentrated hydrochloric acid (HCl). The appearance of red precipitates in the plant sample indicates the presence of Phlobatannins.

13. Test for Ninhydrin

500µL plant extract taken with 500µL Ninhydrin reagent with 5 min. heating, the transformation of plant sample to dark-blue color indicates the presence of Ninhydrin.

14. Test for Anthocyanosides

1mL extract taken in addition with 2mL dilute hydrochloric acid (HCl), the transformation of plant sample to pale pink color indicates the presence of Anthocyanosides.

15. Test for Coumarins

1mL 10% Sodium hydroxide (NaOH) taken with 750µL of plant extract, the transformation of plant sample to yellow color indicates the presence of Coumarins. (Roghini and Vijaylakshmi, 2018; Nathenial et al., 2019).

Isolation and Identification of bacteria

Isolation of bacteria is done to obtain the pure bacterial culture. It was done from raw vegetables, to obtain the best culture, solid Eosin Methylene Blue agar media was prepared as per the below composition per 1lt.: Peptone-10g, Dipotassium phosphate-2g, Lactose-5g, Sucrose-5g, Eosin-0.4g, Methylene blue indicator-0.06g, Agar-15g maintained pH-7.2±0.2 at 25ºC. The following media was prepared and autoclaved at 121ºC for 15 minutes, cooled, and poured in a sterile Petri plate. Spreading of the source was done and incubate for 24 hours at 37ºC. After the 24 hours growth of bacteria, the colony was taken from the spreading plate with a help of a loop, and inoculation of colonies was done by streak plate method.

After the isolation, the identification of bacteria is a very important step for identifying the bacterial species. Identification was done by using conventional methods based on their

characteristics. Hence, the morphological and biochemical test was done for the identification of bacterial genes. Below morphological and biochemical tests were done according to the protocol: Gram staining method, motility test, Starch hydrolysis test, Nitrate Reduction test, Casein Hydrolysis test, Catalase test, Citrate test, H_2S test, Indole test, Methyl Red test, Voges-Proskauer test, Urease test (Aryal, 2019; Saima et al., 2018; Al-Baer and Hussein, 2017).

Antibacterial screening of samples

Antibacterial screening is done to determine the ability of the plant sample extract to inhibit bacterial activity. Here, *Coriandrum sativum* and *Cinnamomum Verum*inhibiting activity wastested by using the disc diffusion method against diarrhea-causing bacteria which was earlier identified as *Shigella* (B1), and *E. coli* (B2). For the test to be carried out Nutrient Agar Media was prepared as per the composition per 1 lt.: Peptone-5g, Sodium Chloride-5g, Yeast extract-3g, and Agar-15 g maintained pH-7.2±0.2 at 25ºC. The following media was prepared and autoclaved at 121ºC for 15 minutes, cooled, and poured in a sterile Petri plate. The negative control disc (where there is no antibiotic) and positive control disc (Ciprofloxacin) were placed in the Petri plate after swapping the bacterial broth on the agar. Similarly, the disc of plant extracts diluted solution was also placed on it. Cultural plates were kept for incubation for 24 hours at 37°C. The diameter (mm) of the zone of inhibition is measured with the help of a ruler. (Razmavar et al., 2014)

RESULT AND DISCUSSION

Table 1 representing the results of qualitative phytochemical tests done on plant extracts showing that Phenols, Flavonoids, Quinones, Terpenoids, Ninhydrin are majorly present in *Coriandrum sativum* whereas Saponin, Tannins, Phenols, Quinones, Terpenoids, Coumarins are at high for *Cinnamomum verum*. The presence of saponins, flavonoids, tannins, coumarins, and terpenoids in plant extract makes it capable of inhibitory action against pathogenic microbes *(*Silva et al., 2016). Hence, the plant extract samples been taken has these phytochemicals in them which justifies that they can inhibit the bacterial action. Saponins, alkaloids, and flavonoids are proven as the most active compound showing antibacterial activity. (Mehreen et al., 2016).

Table 1: Qualitative phytochemical tests results of plant extracts.

S.no.	Qualitative Test	*Coriandrum sativum*	*Cinnamomum verum*
1.	Saponin	-*	+ *
2.	Tannin	-	+ + +
3.	Glycosides	-	+
4.	Phenols	+	+ + +
5.	Steroids	+	+ +
6.	Flavonoids	+ +	+ +
7.	Quinones	+ +	+ + + +
8.	Anthraquinones	-	-
9.	Terpenoids	+ + +	+ + + +
10.	Anthocyanin	-	+/-
11.	Alkanoids	++	+ +
12.	Phlobatannins	-	+ +
13.	Ninhydrin	+ + +	-
14.	Coumarins	+	+ + +
15.	Anthocyanosides	-	+

(+) Positive, (–) Negative, (+/-) May be positive

Table 2 illustrates the bacteria species which has been identified by the morphological and biochemical test. General identification was done and recorded as Gram-negative, motile, positive test for nitrate reduction test, catalase, indole, and Methyl red and negative for others. (Al-Baer and Hussein, 2017; Gebeyehu et al. 2018). The isolated bacterium was suspected as *E. coli*. And Rod-shaped, non-spore-forming gram-negative, non-motile, positive test for nitrate reduction, catalase, and Methyl red (Diriba et al., 2020; Andrews and Jacobson, 2013) bacteria was suspected as *Shigella.*

Table 2: Morphological and Biochemical tests results for identification of bacteria.

S. No.	TEST	B1	B2
1.	Gram-Staining	Gram-negative	Gram-negative
2.	Motility	Non-motile	Motile
3.	Starch hydrolysis	-	-
4.	Nitrate Reduction	+	+
5.	Casein Hydrolysis	-	-
6.	Catalase	+	+
7.	Citrate	-	-
8.	H2S	-	-
9.	Indole	-	+
10.	Methyl Red	+	+
11.	Voges-Proskauer	-	-
12.	Urease	-	-
13.	Possible Bacteria	*Shigella*	*E. coli*

(+) Positive, (–) Negative, (+/-) May be positive

Antibacterial screening results for plant extract samples was done by disc-diffusion method and results were shown in Figure 1 for *Shigella* and *E. coli*. The zone of inhibition was measured including 6mm diameter of discs and recorded in Table 3 and 4. As per the results, graphical representation has been demonstrated for the same in Graph 1 and 2.

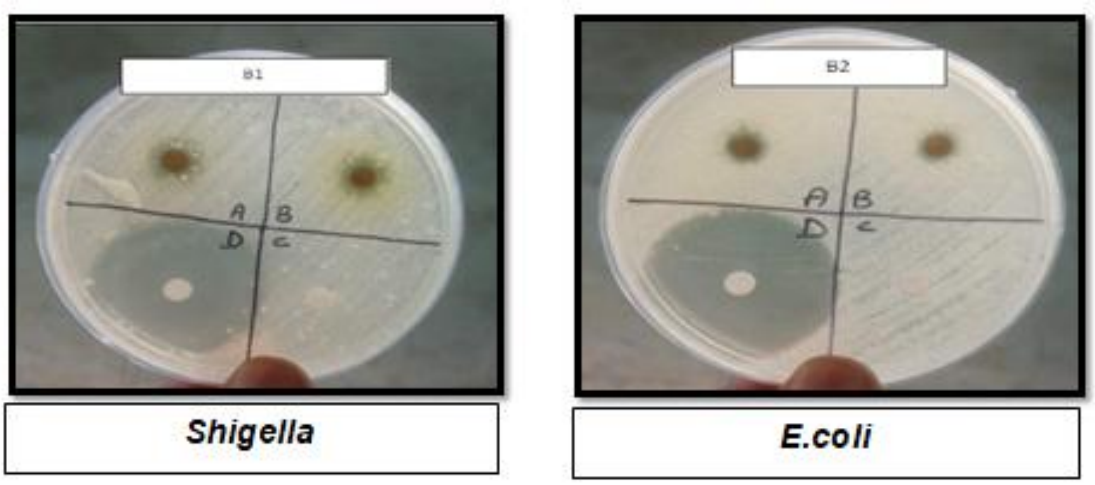


Figure 2: Antibacterial activity of plant samples against identified bacteria.

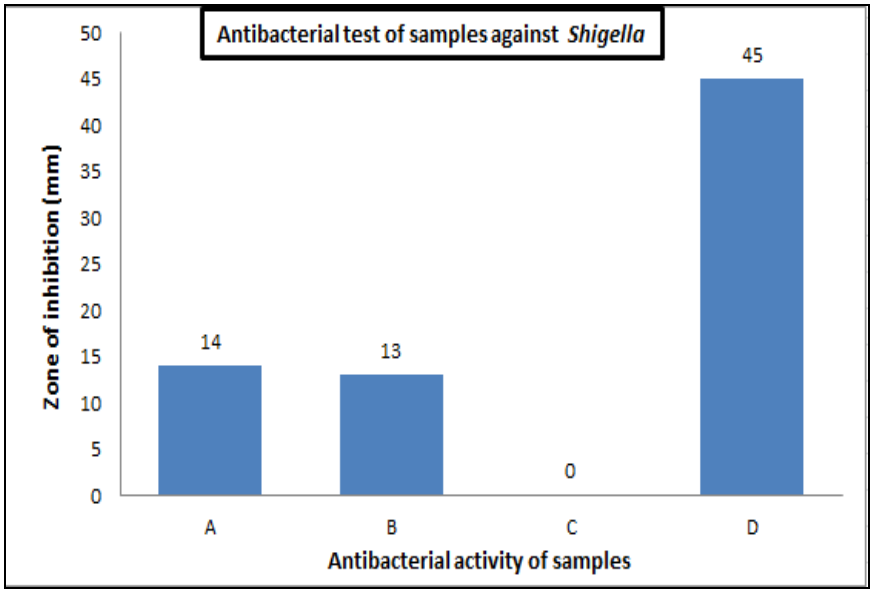
The inhibition zone for *Coriandrum sativum* was 14mm and that of *Cinnamomum verum* was 13mm. The plant sample extracts can able to inhibit the *Shigella* bacterial action. For *E. coli*, the samples *Coriandrum sativum* and *Cinnamomum verum* have inhibition zone of 10mm and 9.5mm respectively. The antibacterial action of samples is low for *E. coli*, but they have the activity to inhibit the bacteria.

Table 3: Inhibition activity of plant samples against *Shigella(Including 6mm diameter of discs).**

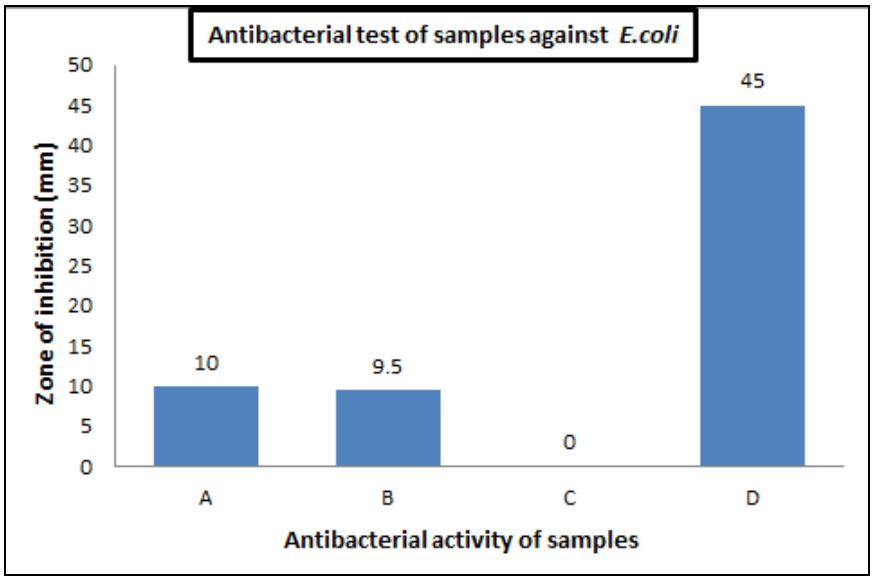
Quadrant	Zone of inhibition (mm)*
A; *Coriandrum sativum*	14
B; *Cinnamomum verum*	13
C; Negative control	0
D; Positive control	45

Table 4: Inhibition activity of plant samples against *E. coli* (*Including 6mm diameter of discs).

Quadrant	Zone of inhibition (mm)*
A; *Coriandrum sativum*	10
B; *Cinnamomum verum*	9.5
C; Negative control	0
D; Positive control	45



Graph 1: Antibacterial activity of samples against *Shigella.*



Graph 2: Antibacterial activity of samples against *E. coli.*

CONCLUSION

The most common and broadly marketed plant samples *Coriandrum sativum* and *Cinnamomum verum* have antimicrobial properties in them so they will truly cooperate for treating any microbe's related disease. In this research, it is concluded that these samples action against diarrhea-causing bacteria *Shigella and E. coli*; which are very common for causing diarrhea, successfully shown their antibacterial activity against the bacteria and created an inhibition zone against them which was observed by the agar disc diffusion method.

Phytochemical qualitative analysis was also done on the samples to get confirm the presence of phytochemicals which will be beneficial for the human body as well as has antimicrobial properties to act against them. Probably the bacteria isolated were identified as *Shigella* and *E. coli* which were suspected after their necessary morphological and biochemical tests. Antibacterial screening results by the disc diffusion method were acceptable; even though both the plant samples showed more dominant effect against *Shigella* than *E. coli*, but as the results are positive against bacteria it can be determined that the samples are effective against the bacteria. Hence, plant samples *Coriandrum sativum* and *Cinnamomum verum* can be further utilized in the future, to treat the diarrhea symptom by developing any nutraceutical product or medicinal product in the form of powder or tablet. Introducing any product formula by incorporating these samples either separately or in the combined form will possibly give relief from diarrhea infections or any other disease caused due to these bacteria. The synergetic effect of plant samples can also be utilized and will possibly show a good result while formulating any product to inhibit the bacterial action.

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