Therapeutic Potential of Native Spices and Herbal Extracts in Modulating *Shigella flexneri* Growth in Gastrointestinal Conditions

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Abstract: Spices and herbs in the sub-continent are considered significantly beneficial in traditional medicines. For ages, people have been using these plant extracts to add flavor to their cooked food as well as for medicinal purposes. We compared the effects of different plants' aqueous extract of 10% concentration with human gut simulated as well as human gut non-simulated conditions on the growth of *Shigella flexneri*, a pathogenic bacterium. In pure culture studies, the growth of *Shigella flexneri* was suppressed in the presence of Cinnamon (*Cinnamonum verum*), Onion (*Allium sepa*), Turmeric (*Curcuma longa*), Garlic (*Allium sativum*), Mint (*Mentha*) and Carom (*Trachyspermum ammi*) extract. On the other hand, Black seeds (*Nigella sativa*) and Coriander (*Coriandrum sativum*) extracts significantly enhanced the growth of *Shigella flexneri*.

However, Fenugreek seeds (*Trigonella foenum*) and Ginger bulb (*Zingiber officinale*) extract neither significantly enhanced nor suppressed the growth of *Shigella flexneri*. The data was observed after 48 hours of incubation at 37°C by taking an optical density of 600nm with the help of a spectrophotometer. Gastrointestinal simulatory conditions were given artificially under a controlled environment, and it was found that the growth of *Shigella flexneri* was decreased, indicating that the consumption of native plant extracts is beneficial in suppressing the growth of the pathogenic bacterium. The indigenous plant extracts exhibited the capacity to inhibit the proliferation of a pathogenic bacterium, specifically *Shigella flexneri*, which ultimately proved beneficial outcomes for maintaining healthy gut microbiota and gastrointestinal ailments.

Keywords: Plant extracts; GIT Simulations; Millipore filter; *Shigella flexneri*; Spectrophotometry.

I. INTRODUCTION

Foodborne illness is a significant issue in developing nations, carrying substantial consequences for public health, trade, and the economy (Akhtar et al., 2014). The employment of numerous preservation methods has not alleviated the concerns of both consumers and the food sector. Food processors, food safety researchers, and regulatory authorities are consistently alarmed by the elevated and increasing incidence of illness outbreaks resulting from certain pathogenic and spoilage bacteria in food (Tauxe et al., 2010). The escalating antibiotic resistance exhibited by certain bacteria linked to foodborne illnesses is a growing issue (Stermitz et al., 2000).

According to (Hernández-Cortez et al., 2017; Mohammad et al., 2018), the majority of food poisoning cases each year are caused by bacteria including *Staphylococcus aureus, Salmonella, Vibrio cholerae, Clostridium perfringens, Shigella, Bacillus cereus*, and *Escherichia coli*. These bacteria are frequently present in numerous uncooked food items. Typically, a considerable quantity of bacteria that cause food poisoning is required to induce illness. Thus, the prevention of disease can be achieved by suppressing the bacterial population, inhibiting their growth, eliminating bacteria using appropriate methods, and developing new techniques. Consequently, there has been a growing fascination with the utilization of natural antibacterial substances, such as spice and herb extracts, for the purpose of their antimicrobial properties as it had long been recognized by humans (Bagamboula et al., 2003). Various plants are utilized to combat diverse ailments and exhibit antibacterial properties (Saleem & Al-Delaimy, 1982). However, with the evolving globe, there has been a growing consumer desire for natural goods. People in Asia, particularly South Asia, have utilized spices for various purposes, such as adding color to their cooked food or enhancing its scent (Sachan et al., 2018). However, certain herbs and spices can inhibit the proliferation of harmful microorganisms and possess therapeutic qualities that can reduce symptoms or prevent illness (Lai & Roy, 2004). According to Arora and Kaur (1999), the presence of resistant strains has prompted researchers to discover novel medications, and it has been found that specific native plants do possess antibacterial capabilities.

A recent study by Kalemba and Kunicka (2003) found that clove, cinnamon, black cumin, and garlic exhibit strong antibacterial properties against *Bacillus subtilis*, *E. coli*, and

Saccharomyces cerevisiae. Various researchers from worldwide have also reported the inhibitory activity of spices. Moreover, crude extracts derived from black cumin (*Nigella sativa*) have shown antibacterial efficacy against various bacterial strains (Morsi, 2000).

After conducting extensive research on the antibacterial properties of garlic, Cavallito and Bailey (1944) concluded that certain bacteria were completely incapable of developing resistance to allicin and crushed garlic as some of its derivatives have antimicrobial activities.

Cinnamon (*Cinnamomum verum*) and (*Cinnamomum zeylanicum*), is a members of the *Lauraceae* family. This plant is extensively utilized in medicinal industries due to its antioxidant, antibacterial, and anticarcinogenic properties. Furthermore, antibacterial activities are also shown by cinnamon oil against *E. coli, Salmonella, Bacillus* and *S.aureus* (Azad et al., 2018).

Turmeric, scientifically known as *Curcuma longa*, is a traditional herbal remedy. It is a member of the *Zingiberaceae* family. The presence of curcumin, a polyphenolic component, in turmeric extracts has been found to exhibit antibacterial and antioxidant properties (Irshad et al., 2018). Hence, the antioxidant properties of curcumin can be attributed to its phenolic component (Sharma et al., 2019).

Ginger, derived from the rhizome of *Zingiber officinale,* is indigenous to Asia and has been utilized globally as a medicinal remedy for over two millennia (Erasmus & Leisegang, 2021). Ginger comprises polyphenolic constituents such as phenolic acids. The primary components of this substance exhibit several biological properties, including antioxidant, antidiabetic and antibacterial effects (Singh et al., 2018; Singh & Singh, 2019).

Mint has been utilized in diverse domains, including medications and cosmetics. The aqueous extracts of mint possess antioxidant capabilities as a result of the presence of phenolic components (Diniz do Nascimento et al., 2020). The anti-inflammatory properties of mint essential oil make it a viable short-term alternative treatment for irritable bowel syndrome in humans (Chumpitazi et al., 2018). Multiple studies have demonstrated the plant's inhibitory capacity, which varies depending on the bacteria type. Additionally, it exhibits potent antibacterial properties against gram-positive bacteria, particularly *S. aureus*. Additional research has documented the varying amounts of oil from this plant and its antibacterial properties (Stringaro et al., 2018).

However, there is very little amount of information present about the antimicrobial properties of herbal extracts on the pathogenic bacterium *Shigella flexneri*. This study endeavors to evaluate the antimicrobial efficacy of various spices and herbal extracts, particularly on *Shigella flexneri*, by providing artificial gastrointestinal simulatory conditions and by comparing it with non-simulatory conditions

II. MATERIAL AND METHODS

Assorted specimens of various therapeutic herbs and spices were gathered from the local markets of Lahore, Pakistan. All compounds utilized in the investigation were of analytical grade. The readings were taken three times, with subsequent reporting of the mean values (Liu et al., 2010).

A. Preparation of Spices and Herbs

10g of each spice and herb, including mint, garlic, ginger, cinnamon, black seeds, fenugreek seeds, onion, carom, and coriander, were powdered and placed in pre-weighed culture bottles (Atanda et al., 2007). The substrate was then suspended in 100ml of distilled water.

B. Preparation of Aqueous Extract under Non-Gastrointestinal Conditions

The culture bottles with solution were left in a dark place for three days and the suspension was filtered by using Whatman No. 1 filter paper. The filtered material was then subjected to drying in an oven at a temperature of 50°C. After that, the residue was weighed, and the volume was raised by adding an appropriate amount of distilled water to make an aqueous extract of 10% concentration (w/v) solution. Subsequently, the solution underwent centrifugation, and the resulting supernatant was processed through a Millipore filter (Syringe filter MILLEX-OR, 0.22μm, 25mm, sterile) and stored in autoclaved vials at 4°C in the refrigerator (Zhu et al., 2013).

C. Preparation of Aqueous Extract under Gastrointestinal Conditions

The identical methodology was replicated as described earlier, this time employing simulated fluids for the treatment of the spices. Minekus et al. (2014) protocol was used but with little modification. The extracts were prepared using the steps mentioned earlier and subsequently filtered through a Millipore filter (Syringe filter MILLEX-OR, 0.22μm, 25mm, sterile) was stored in autoclaved vials at 4°C in the refrigerator (Zhu et al., 2013).

D. Isolation of Bacterial Strains

Samples were collected with the help of the swab method and stored in a saline solution at 37°C for 48 hours. For the purposes of detection, isolation and enumeration, eosin-methylene blue agar was used (Ema et al., 2022). Three dilutions were made before performing quadrant-streaking on the plate and leaving it in the incubator at 37°C for a period of 48 hours (Marajan et al., 2018). The bacterial colonies were observed and an isolated colony were taken, re-streaked and different identification tests were performed, and it was identified as *Shigella flexneri* (Kuzina et al., 2001).

E. Effects of Aqueous Extracts on Shigella flexneri Growth under Non-Gastrointestinal Conditions

Nutrient broth media was prepared and autoclaved. However, 5 ml of media were taken in each vial. The first vial was taken, and 0.1 ml of inoculum was inoculated by removing the same quantity of media. In the second vial, 50 μl of media solution was removed, and 50 μl of a spice extract was added. In the third vial, not only was 50 μl of media removed, but also 0.1 ml of solution was withdrawn. Subsequently, 0.1 ml of bacterial inoculum was introduced, followed by the addition of 50 μl of spice extract, thoroughly blended together.

Furthermore, the fourth vial was taken as a blank. The same procedure was followed for all the ten spices. Consequently, the vials were placed in an incubator at 37°C for 48 hours, and optical density was taken at 600nm with the help of spectrophotometry. The experiment was performed in triplicates (Abbas et al., 2014).

F. Effects of Aqueous Extracts on Shigella flexneri Growth under Gastrointestinal Conditions

Each spice aqueous extract was taken, which was already treated with simulatory conditions by using the (Minekus et al., 2014) protocol with little modification. A similar procedure was followed, and samples were stored at 37°C incubator for 48 hours. Optical density at 600nm was set and reading was noted with the help of spectrophotometry (O'Mahony & Papkovsky, 2006). Samples were taken in triplicates.

G. Data Analysis

The analysis of variance was conducted using the two-way analysis of variance (ANOVA) approach. The SPSS was utilized to compute the statistically significant differences when the *p*-value was equal to 0.05. All results were expressed as Mean ± Standard deviation (SD) (Hazra & Gogtay, 2016).

III. RESULTS

The *Shigella flexneri* growth is taken as a control in nutrient broth and compared with 10 different spices and herbal extracts under gastrointestinal simulatory conditions and non-gastrointestinal simulatory conditions.

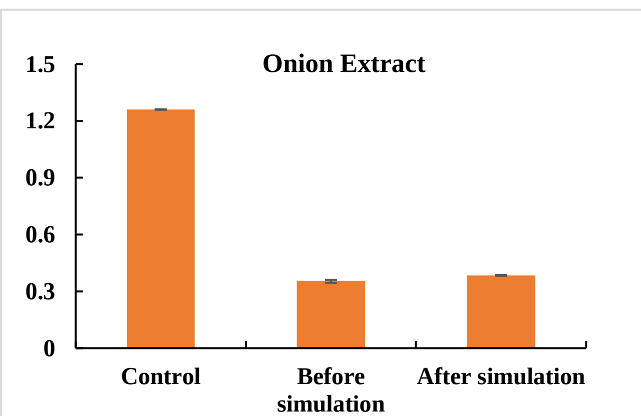
The growth of *Shigella flexneri* was suppressed in the presence of cinnamon extract by 70%, onion extract by 69%, turmeric extract by 66%, garlic extract by 37%, Carom extract by 12 % and mint extract by 15% overall in both gastrointestinal and non-gastrointestinal conditions as compared to its control growth.

On the other hand, black seed extract enhanced the growth of *Shigella flexneri* by 21% and coriander extract by 3%. However, fenugreek seeds and ginger bulb extract also did not significantly suppress or enhance the growth of *Shigella flexneri* under gastrointestinal simulatory and non-gastrointestinal simulatory conditions.

Table 1: Effect of aqueous extract of different spices and herbs on the growth of *Shigella flexneri* under non-simulatory and simulatory conditions.

Sr. No.	Spices (aqueous extract)	Control (*Shigella flexneri*)	Before-Simulation (*Shigella flexneri*)	After-Simulation (*Shigella flexneri*)
1	Cinnamon	1.26±0.001	0.370±0.003	0.448±0.002
2	Turmeric	1.26±0.001	0.425±0.013	0.430±0.001
3	Onion	1.26±0.001	0.355±0.006	0.348±0.001
4	Garlic	1.26±0.001	0.794±0.005	0.834±0.002
5	Mint	1.26±0.001	1.067±0.001	1.055±0.000
6	Carom	1.26±0.001	1.101±0.003	1.178±0.002
7	Black seeds	1.26±0.001	1.495±0.003	1.573±0.001
8	Coriander	1.26±0.001	1.177±0.003	1.378±0.001
9	Fenugreek seeds	1.26±0.001	1.381±0.001	1.573±0.001
10	Ginger	1.26±0.001	1.481±0.001	1.537±0.002

*Mean values are the results of three triplicates.



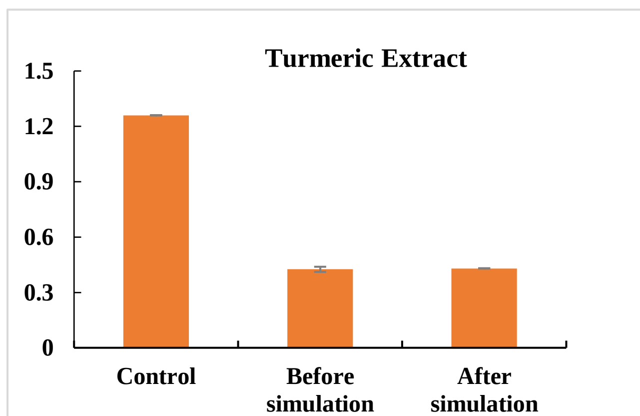
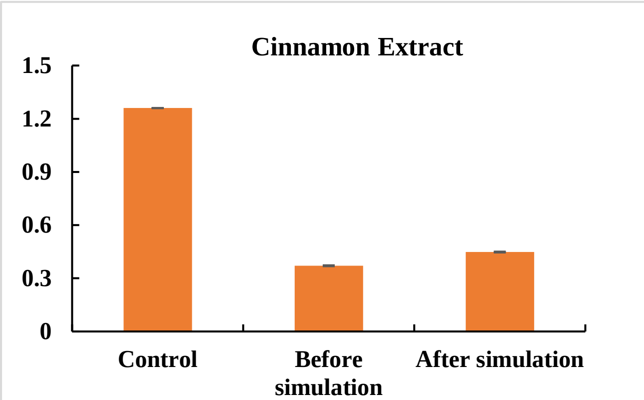


Figure 1, 2: Effect of Cinnamon and Onion extract showing a visual decrease in the growth of *Shigella flexneri* in simulatory conditions as well as non-simulatory conditions while comparing with control



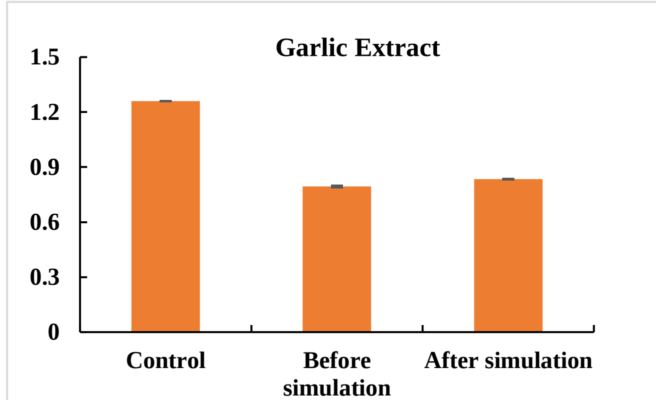
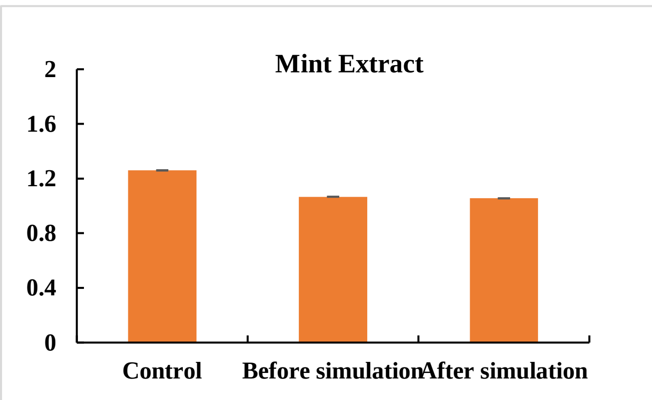


Figure 3, 4: Effect of Turmeric and Garlic extract showing a visual decrease in the growth of *Shigella flexneri* in simulatory conditions as well as non-simulatory conditions while comparing with control



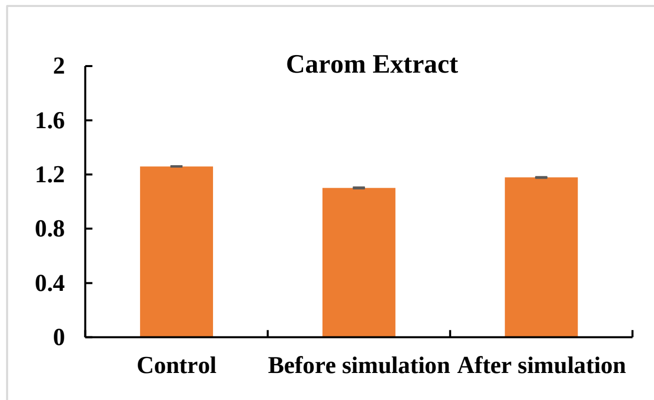
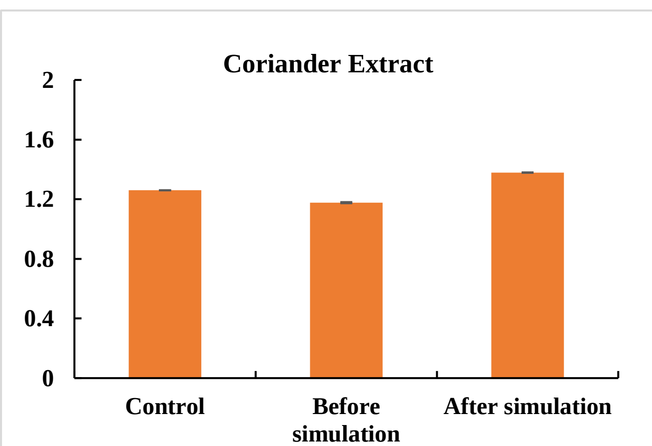


Figure 5, 6: Effect Carom and Mint extract showing a visual decrease in the growth of *Shigella flexneri* in simulatory conditions as well as non-simulatory conditions while comparing with control



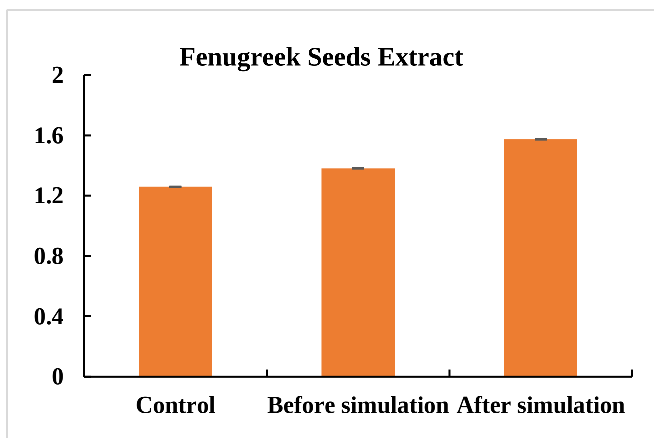
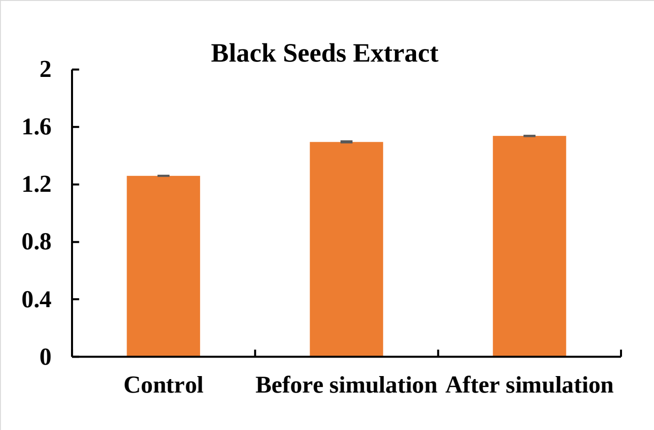


Figure 7, 8: Effect of Black seeds and Coriander extract showing a visual increase in the growth of *Shigella flexneri* in simulatory conditions as well as non-simulatory conditions while comparing with control



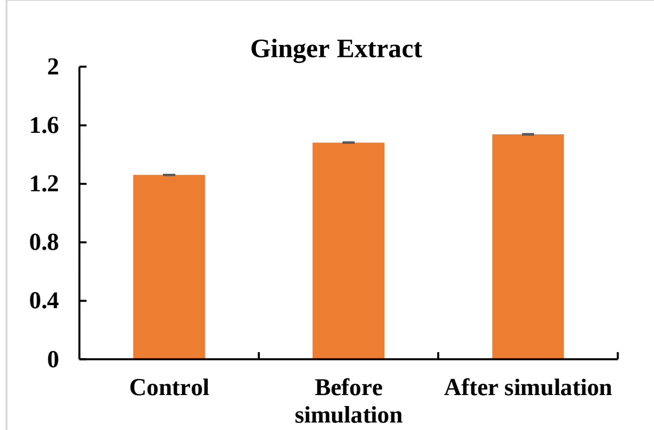


Figure 9, 10: Effect of Fenugreek seed and Ginger extract did not significantly suppress or enhance the growth of *Shigella flexneri* in simulatory conditions as well as non-simulatory conditions while compared with control

IV. Discussion

The effects of spices and herbs were examined to see if they had any effect on the growth of the pathogenic bacterium *Shigella flexneri*. The study also sought to determine whether these substances could inhibit the growth of *Shigella flexneri* and indirectly promote the proliferation of a healthy gut microbiota. This evaluation was conducted under both gastrointestinal simulatory and non-gastrointestinal simulatory conditions. The optical density was taken at 600nm with the help of spectrophotometry, and it was proved that the growth of pathogenic bacterium was suppressed in the presence of a few spices. However, similar results were also observed by Munir et al. (2023) when the growth of probiotics was supported as it can also help to suppress the growth of *Shigella spp*.

According to Bajpai et al. (2008), spices and herbs can serve as a substitute for preservatives and be employed as a strategy to control pathogens in food products. Plant-derived antimicrobials can be found in a diverse range of plants, spices, and herbs. Spices and herbs have the dual goal of enhancing taste and extending shelf life. Spices and herbs, initially incorporated to enhance flavor, can also organically and securely extend the longevity of food products (Holley & Patel, 2005). Multiple techniques exist for evaluating the antibacterial properties of spices, herbs, and their constituents. These methods have a significant impact on the level of inhibition seen. There is variation in the activity against microorganisms depending on the specific spices and herbs used, either alone or in combination (Radušienė et al., 2007). While the current study's findings are promising, it would be hasty to make any definitive conclusions based solely on these data. Additional investigation is required to determine the practicality of utilizing whole dehydrated spices and herbs in food production to manage the proliferation and viability of the *Shigella flexneri* bacterium (El-Shamy et al., 2013).

V. Conclusion

The present study's findings establish the hierarchy of antibacterial efficacy among the examined herbs as follows: Cinnamon > Onion > Turmeric > Garlic > Carom > Mint. However, these herbs can be chosen as potentially effective antibacterial agents in food products based on the desired flavor of the product. Available data from current and prior research indicates that herbs and spices have significant potential as an antibacterial agent and could be suggested for usage, ideally in combination. These discoveries could potentially result in numerous advantageous outcomes, including a decrease in the utilization of antibiotics. Consequently, this would lead to a reduction in the emergence of antibiotic resistance among harmful microbes.

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Conflict Of Interest

The authors declare that they have no conflict of interest.

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