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In-vitro Study of the Medicinal Properties of *Coriandrum Sativum* and *Laurus Nobilis* against *Escherichia coli* and *Bacillus* species.

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ABSTRACT

Antibiotic toxicity is the most prominent challenge that is being faced by the medical world today. Herbs and spices, used for thousands of years to enhance the flavor, color and aroma of food, are being increasingly used to mitigate this problem by means of their preservative and medicinal values. Natural antibiotic products reduce the microbial resistance and provides cure without eliciting any side effects. This study is based on the antimicrobial activities of *Coriandrum sativum* (coriander) and *Laurus nobilis* (bay leaves) against *Escherichia coli* and *Bacillus* species. These activities were analyzed and compared with tetracycline, as a reference antibiotic. Antimicrobial activity was measured using the Agar well diffusion method. The results obtained indicated that *Coriandrum sativum* and *Laurus nobilis* have medicinal properties.

Keywords: Antimicrobial Activity, Diffusion, Zone of Inhibition, Antibiotics, *Bacillus*, *Escherichia coli*

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INTRODUCTION

Spices have been used for many centuries by various cultures to enhance flavor and aroma of our foods. The usage of spices in food preservation and in treatment of clinical ailments is well recognized and there are several reports on the development of antibiotic resistance in diverse bacterial pathogens [1]. At present, it has been estimated that about 80% of the world population rely on botanical preparations as medicine to meet the needs as they are considered safe and regarded to be effective against certain ailments [2]. There has been increasing interest in the development of new types of effective and nontoxic antimicrobial compounds. Attention has been focused on using natural antibacterial compounds such as extracts of spices and herbs for food preservation [3]. Spices are some of the most commonly used natural antimicrobial agents in foods. Addition of spices in foods not only imparts flavor and pungent stimuli but also provides antimicrobial property [4]. The unique aroma and flavor in spices are derived from compounds known as phytochemicals or secondary metabolites [5]. The phytochemicals are antimicrobial substances present in the spices which are capable of repelling harmful organisms. They also serve as photoprotectants and responds to environmental changes effectively. Numerous classes of phytochemicals including the isoflavones, anthocyanins and flavonoids have been found to be associated with the spices [6]. Coriander has traditionally been referred to as antidiabetic [6], anti-inflammatory and cholesterol lowering [7]. Research also suggests that the volatile oils found in the leaves of *C. sativum* plant may have antimicrobial properties against food borne pathogens such as *Salmonella* species [8]. Bay leaf oil tested for its bactericidal activity has shown to be active against *Salmonella enteric* and *E. coli* [9]. The present study focuses on the antibacterial properties of *Coriandrum sativum* and *Laurus nobilis*.

MATERIAL AND METHODS

Isolation of microorganisms

Sewage samples were collected for the isolation of microorganisms from the sewage treatment plant located at Lovely Professional University. The samples were then diluted to 10^{-1} to 10^{-15} and the diluted samples were spread on Nutrient agar plates in order to get pure colonies of bacteria after incubating the plates at 37°C for 24 hours. Colonies differing in morphological characteristics were selected and streaked on selective media like EMB and Mannitol medium. The plates were then incubated at 37°C for 48 hours [10].

Characterization of microorganisms

The types of selected isolates were identified by biochemical and morphological tests of Bergey's Manual of Determinative bacteriology. Morphology of isolates was examined under optical microscope by gram staining. Biochemical properties of isolates were analyzed by Indole test, Methyl Red test, Voges Proskauer test, Citrate Utilization test, Catalase test, Sucrose Fermentation test, Lactose Fermentation test, Glucose Fermentation test, Reduction of Nitrate test and Starch Hydrolysis test [10].

Collection of Spices

Spices were collected from authorized Ayurvedic stores. Both the spices were authenticated and identified by reputed botanists.

Preparation of extracts

Fresh coriander and bay leaves were oven dried at 70°C for 2 hours and crushed to make fine powders using mortar and pestle. The extracts of coriander and bay leaves were prepared separately by using 5 gm of the respective powders in 50 ml of distilled water heated to 70°C using soxhlet apparatus. The obtained extracts were stored in sterile bottles at 4 °C after weighing [11].

Determination of antimicrobial activity

The antimicrobial activity of aqueous extracts of *Coriandrum sativum* and *Laurus nobilis* was checked against two microorganisms, *E.coli* and *Bacillus* species by Agar well diffusion method. Muller Hinton agar was prepared by mixing it with distilled water and sterilizing it in autoclave. Petri plates containing the

Nutrient medium were inoculated with bacterial culture (*Escherichia coli* and *Bacillus* species). In order to check antimicrobial activity of extracts at various concentrations, extracts were diluted by adding varying amount of distilled water (Table 1). After dilution, 10 µl of each extract dilutions were filled in the wells and the petri plates were incubated at 37°C for 24 hours. Antibiotic disc of tetracycline was also placed in a petri plate that was considered as the control. The zones of inhibitions, formed in this way, were measured and analyzed.

Table 1: Percentage dilution of extracts; DW = distilled water.

Dilutions	Percentage of Extract (%)
Extract	100.00
750µl extract + 500µl DW	060.00
250µl extract + 250µl DW	050.00
500µl extract + 500µl DW	050.00
500µl of above dilution + 500µl DW	025.00
500µl of above dilution + 500µl DW	012.50
500µl of above dilution + 500µl DW	006.25

RESULTS AND DISCUSSION

Identification of bacterial species

The sensitivity of bacterial species towards the antimicrobial components present in the spices is a characteristic of the microorganisms. In this work, bacterial strains were isolated from sewage samples. Out of them two isolates were screened from initial level. The screening of the isolates was conducted on the basis of their biochemical and morphological properties (Table 2). The two bacterial isolates were analyzed as follows: Isolate 1 was *E.coli*, Isolate 2 was *Bacillus* species (Fig.1).

Table 2: Biochemical and morphological tests applied to confirm the presence of the desired microorganisms. + shows that the microorganisms gave a positive result and – shows that the microorganisms gave a negative result.

Tests	<i>E. coli</i>	<i>Bacillus sp.</i>
Grams Staining	-	+
Indole	+	-
Methyl Red	+	-
Voges Proskauer	-	+
Citrate Utilization	-	+
Catalase	-	+
Sucrose Fermentation	+	+
Lactose Fermentation	+	-
Glucose Fermentation	+	-
Reduction of Nitrate	+	+
Starch Hydrolysis	-	+



Figure 1: Isolated microorganisms: (a) *E. coli*; (b) *Bacillus*.

Antimicrobial assay

Agar well diffusion assay was performed in order to check the antimicrobial activity of spices against *E.coli* and *Bacillus* species with respect to different concentration of the extracts. The results are shown in Table 3. All extracts of Coriander and Bay leaves showed zones of inhibition against *E.coli* and *Bacillus* species. The aqueous extracts of coriander and Bay leaves were more effective against *Bacillus* species than *E.coli* because they depicted higher zones of inhibition against *Bacillus* species (Figs. 2-5). In comparison to both spices, Bay leaves showed a wider zone of inhibition against both the bacteria compared to coriander (Fig.6). The results suggested that these spices have inhibitory effects against bacteria and can be used for various purposes in the field of medication for the welfare of the mankind. These spices can be used for the treatment of infectious diseases and also help in the development of new drugs, thus providing an effective source of herbal medication.

Table 3: Antimicrobial activity assay with the diameters of the zone of inhibition

Percentage of Extract (%)	Diameter of zone of inhibition formed against <i>Bacillus</i> by Coriander (mm)	Diameter of zone of inhibition formed against <i>Bacillus</i> by Bay leaf (mm)	Diameter of zone of inhibition formed against <i>E.coli</i> by Coriander (mm)	Diameter of zone of inhibition formed against <i>E.coli</i> by Bay leaf (mm)
100.00	20	20	11	16
060.00	15	15	9	11
050.00	13	12	6	8
050.00	14	13	8	10
025.00	13	12	5	8
012.50	12	11	0	6
006.25	11	10	0	5
Control	17	14	18	15

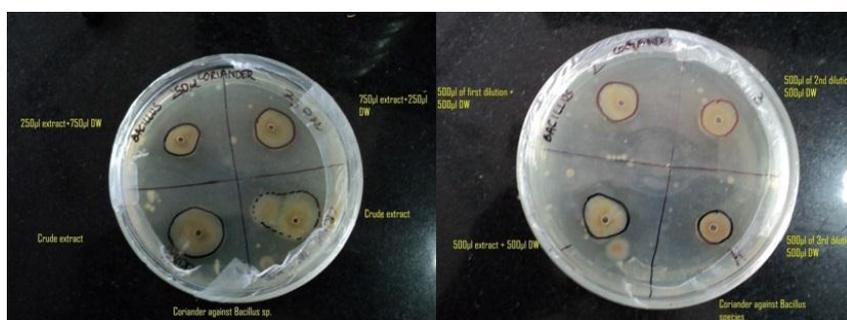


Figure 2: Zones of inhibition observed against *Bacillus* species by Coriander.

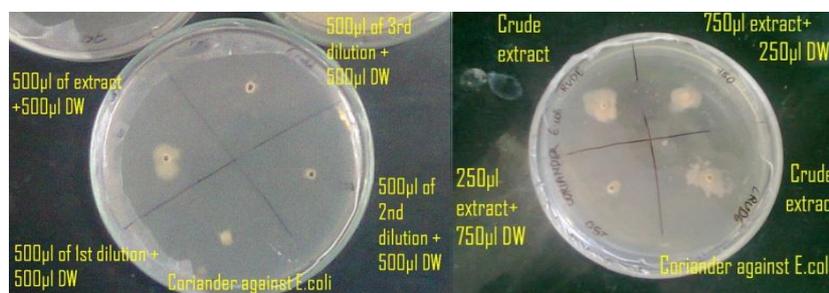


Figure 3: Zones of inhibition observed against *E.coli* by Coriander

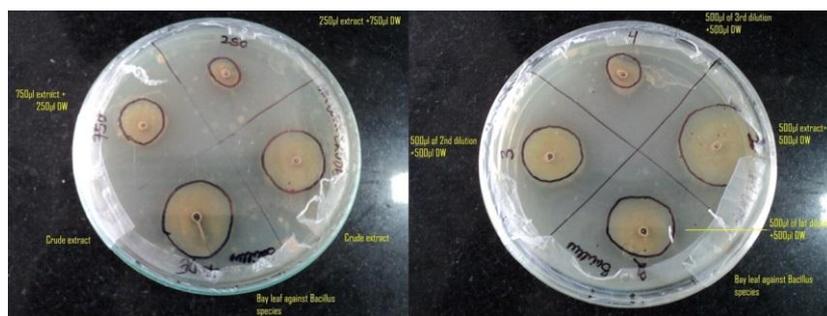


Figure 4: Observed zone of inhibition formed by Bay leaf against *Bacillus* species.



Figure 5: Observed zone of inhibition formed by Bay leaf against *E. coli*

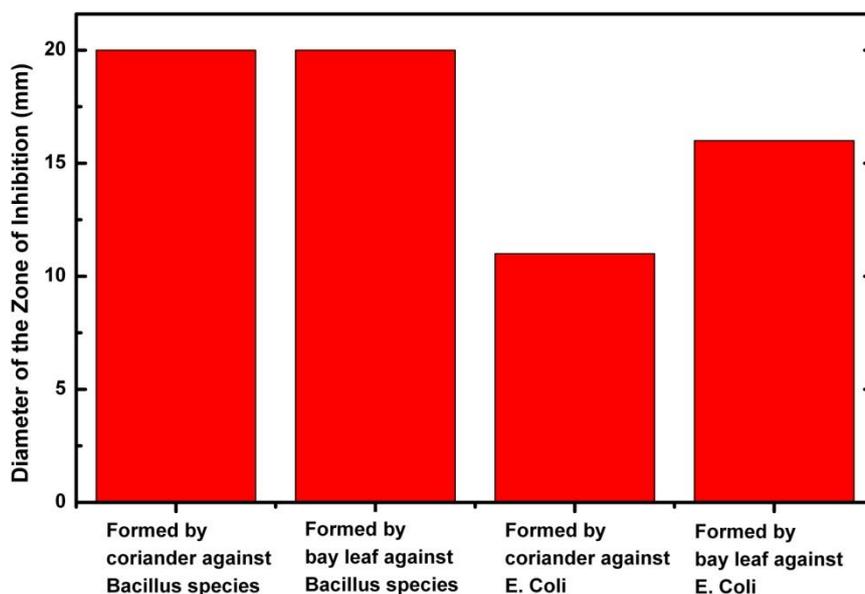


Figure 6: Plot showing diameters of inhibition zones obtained from 100% aqueous extracts of *Coriandrum sativum* (coriander) and *Laurus nobilis* (bay) against *Bacillus* species and *E. coli*.

CONCLUSION

Natural herbs and spices extracts could be used for their remedial properties like antibacterial and antifungal actions. In this work, it was observed that the aqueous extracts of Coriander and Bay leaves could mitigate tested microorganisms (*E. coli* and *Bacillus*). These extracts depicted a zone of inhibition against the isolated microorganisms. Therefore, the antimicrobial components present in the Coriander and Bay leaves could be used in the development of active drugs in the developing pharmaceutical industries.



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