

## Bactericidal Activity of Black Pepper, Bay Leaf, Aniseed & Coriander Against Clinical Isolates

Fasiha Saeed <sup>1\*</sup>, Naila Kanwal <sup>1</sup>, Sayyada Ghufrana Nadeem <sup>2</sup>, Shazia Tabassum Hakim <sup>3</sup>,

<sup>1</sup> Department of Microbiology Jinnah University for Women, Karachi, Pakistan.

<sup>2</sup> Medical Mycology Research And Reference Laboratory, Department of Microbiology, Jinnah University for Women, Karachi -74600, Pakistan.

<sup>3</sup> Virology and Tissue Culture Laboratory, Department of Microbiology, Jinnah University for Women, Karachi -74600, Pakistan.

### ABSTRACT

Since ancient times spices and herbs have been used as food additives and also as flavoring agents and possess antimicrobial activity against different micro organisms and food pathogens. Different factors influence the antimicrobial activity of spices including type of spices or herbs, their chemical composition, content of extracts and essential oils and the type of micro organisms against they are used. The present study was evaluated to determine the antimicrobial potential of spice extracts of Black pepper (*Piper nigrum*), Coriander (*Coriandrum sativum*), Aniseed (*Pimpinella anism*) and Bay leaf (*Laurus nobilis*) against Gram positive and Gram negative microorganisms. The study was performed disc diffusion method, the extract of Black pepper, Coriander, Aniseed and Bay leaf exhibits antimicrobial activity against all tested microorganisms with an inhibition zone diameter (IZD) ranging from 22mm to 16mm. The inhibitory effect was found in all tested microorganism except against *Pseudomonas* which shows resistance to spice extract.

**Keywords:** Black pepper (*Piper nigrum*), Aniseed (*Pimpinella anism*), Coriander (*Coriandrum sativum*) and Bay leaf (*Laurus nobilis*).

### INTRODUCTION

In daily life humans have a long history of using spices and herbs as a food flavoring, food preservation and for medicinal purposes ( Benefits of Bayleaf, 2012; Davidson, 2010). Spices is derived from the Latin "species aromatacea", meaning fruits of the earth and are defined as an "aromatic, pungent vegetable substance. Spice sources include bark, bulbs, buds, flowers, fruit leaves, roots, seeds and plant tops (Rosen, 2012). Spices are recognized to stabilize the foods against microbial deterioration. This could be observed when spices show initially high microbial charge and as time progresses, the microbial growth become progressively slower or it

\*Corresponding author: fasih37@hotmail.com

is eventually totally suppressed (Kizil, and Sogut, 2003). Aniseed (*Pimpinella anisum*): *Pimpinella anisum* L., one of the oldest medicinal plants belonging to the Umbelliferae family. Aniseeds contain 1.5–5% essential oil and used as flavorings, digestive, carminative, and relief of gastrointestinal spasms (Zargari, 1996). According to Asuzu, an acetone extract of aniseed inhibited the growth of a variety of bacteria counting *Escherichia coli* and *Staphylococcus aureus*, and also (Asuzu IU, 1986) the essential oil of aniseed exhibited strong antifungal activity against yeasts and dermatophytes, followed by *C. albicans*, *C. glabrata*, and *Geotrichum spp.* (Kosalec *et.al.*, 2005). Coriander (*Coriandrum sativum*), first is called "coriandrum". The countries of its origin are the shores of the Mediterranean and

Central Asia, and it is cultivated all over the world (Du,1999).The essential seed oil is used in various herbal remedies and dietary supplements, and to flavor, vermouth, liqueurs, tobacco and perfumery. In 2001 Elgayyar *et al.* and Larran *et al.* studied *Coriandrum sativum* and observed that the essential oil of coriander inhibited microorganisms. Black pepper (*Piper nigrum*): Pepper plant is widely cultivated throughout Indonesia belongs to Pepper family Piperaceae.. It may reach heights of 10 m (33 feet), broad, shiny green leaves are alternately arranged. The berrylike fruits, or peppercorns, are about 5 mm (0.2 inch) in diameter. They become yellowish red at maturity and bear a single seed. Their odor is penetrating and aromatic; the taste is hot, biting, and very pungent (Black pepper, 2012). Pepper exhibits Antispasmodic, Anti-toxic, Carminative, Digestive, Diuretic, Muscle Relaxant like medicinal properties. In 2006 Masood, N., A. Chaudhry and P. Tariq found black pepper had antibacterial activity against *P. aeruginosa*, *S. aureus* and *E. coli*. (Ertürk, 2006). Black pepper was found to be effective against Salmonella and *Bacillus subtilis* (Reddy *et.al.*, 2001). Bayleaf (*Laurus nobilis*): *Laurus nobilis* L., also called bay laure, one of the most well-known and most frequently used plants from The Lauraceae family. The Lauraceae family occurs within the subtropics and tropics of Eastern Asia, South and North America. The chemical composition of bay leaves has been extensively studied. Its essential oil, also called laurel leaf oil (Rohwer *et.al.*, 1993). known to provide resistance against different types of cancer. It contains caffeic acid, eugenol and catechins, all of which posses chemo-protective properties. Hammer *et.al* (Hammer *et.al.*,1999) found that bay leaf extract inhibit the growth of *Staphylococcus aureus* (Al-Hadi, 2011).

## MATERIALS AND METHODS

*Study Area & Design:* The study was conducted in

Jinnah University for Women Nazimabad, Karachi, Pakistan between July'2012 to December'2012.

*Sampling:* 25 clinical isolates belongs to 6 different genera *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aerogenosa* was used in study.

*Extract Preparation:* Four Herbal spices, aniseed (*Pimpinella anism*), coriander (*Coriandrum sativum*), black pepper (*Piper nigrum*) and bay leaf (*Laurus nobilis*), were collected locally. Aqueous extract of aniseed, coriander seed, black pepper seed and bay leaf were prepared by boiling 10g in 100ml sterile distilled water over low flame for 45 minutes. The flasks were then plugged and remove from heat and allow cooling. After cooling the content of flasks were filtered.

*Inoculum Preparation:* To prepare inoculums four to five colonies from pure culture of each test organism were transferred to 5ml Nutrient broth. The broth was incubated at 37°C for 24 hours. The turbidity of culture was compared with 0.5 McFarland Nephelometer standards to get  $150 \times 10^6$  CFU/ml.

*Protocol:* Screening of antibacterial activity was performed by disc diffusion technique used by Chaudhry N.M in 2006 with some modifications. For disc diffusion method discs of Whatmans No .1 filer paper having a diameter of 6mm were placed in sterile Petri plate wrapped in paper and oven dried at 65°C for 1 hour for sterilization. The inoculums were inoculated on MHA plates with the help of sterile cotton swab. Then sterile discs with absorb spice extract (75µl/disc) were placed on the agar by pressing gently (Masood, 2006). After that plates were incubated at 37°C for 24 hours. Observe zones of inhibition and calculated the mean zones of inhibition.

## RESULTS

**Table I:** Antibacterial activities of extract of black pepper (*Piper nigrum L.*), bay leaf (*Laurus nobilis L.*), aniseed (*Pimpinella anisum L.*) and coriander (*Coriandum sativum L.*) against microbial flora isolated from clinical samples.

S.No	Microorganisms	Diameter of Zone of inhibition {to the nearest millimeter(mm)}			
		Aniseed	Black pepper	Coriander	Bay leaf
1	<i>Escherichia coli</i>	14±0.1mm	23±0.1mm	14±0.1mm	12±0.3mm
2	<i>Escherichia coli</i>	11±0.3mm	20±0.1mm	13±0.2mm	-
3	<i>Escherichia coli</i>	-	23±0.1mm	13±0.2mm	11±0.3mm
4	<i>Escherichia coli</i>	12±0.3mm	22±0.1mm	-	10±0.3mm
5	<i>Escherichia coli</i>	11±0.3mm	-	12±0.3mm	11±0.3mm
6	<i>Staphylococcus aureus</i>	16±0.1mm	20±0.1mm	12±0.3mm	11±0.3mm
7	<i>Staphylococcus aureus</i>	14±0.1mm	-	11±0.3mm	-
8	<i>Staphylococcus aureus</i>	-	16±0.1mm	11±0.3mm	11±0.3mm
9	<i>Staphylococcus aureus</i>	14±0.1mm	14±0.1mm	10±0.3mm	-
10	<i>Staphylococcus aureus</i>	12±0.3mm	14±0.1mm	-	11±0.3mm
11	<i>Klebsiella pneumonia</i>	-	12±0.3mm	-	11±0.3mm
12	<i>Klebsiella pneumonia</i>	10±0.3mm	12±0.3mm	14±0.1mm	-
13	<i>Klebsiella pneumonia</i>	12±0.3mm	-	12±0.3mm	11±0.3mm
14	<i>Pseudomonas aeruginosa</i>	-	-	-	-
15	<i>Pseudomonas aeruginosa</i>	-	-	-	-
16	<i>Pseudomonas aeruginosa</i>	-	-	-	-
17	<i>Pseudomonas aeruginosa</i>	-	-	-	-
18	<i>Bacillus subtilis</i>	12±0.3mm	12±0.3mm	10±0.3mm	11±0.3mm
19	<i>Bacillus subtilis</i>	10±0.3mm	10±0.3mm	-	11±0.3mm
20	<i>Bacillus subtilis</i>	-	11±0.3mm	14±0.1mm	-
21	<i>Bacillus subtilis</i>	14±0.1mm	-	12±0.3mm	11±0.3mm
22	<i>Proteus mirabilis</i>	-	15±0.1mm	12±0.3mm	14±0.1mm
23	<i>Proteus mirabilis</i>	14±0.1mm	-	11±0.3mm	12±0.3mm
24	<i>Proteus mirabilis</i>	10±0.3mm	12±0.3mm	-	-
25	<i>Proteus mirabilis</i>	12±0.3mm	12±0.3mm	10±0.3mm	-

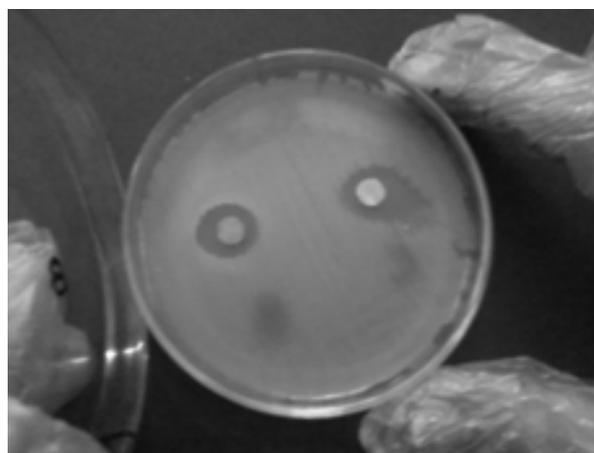
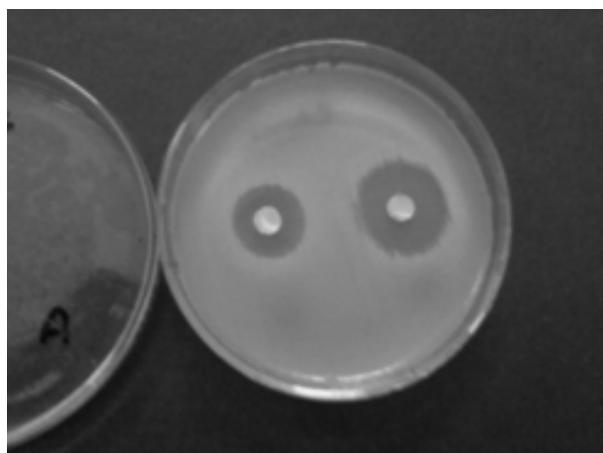
**Table II:** Mean zones of inhibition of extract of black pepper (*Piper nigrum L.*), bay leaf (*Laurus nobilis L.*), aniseed (*Pimpinella anisum L.*) and coriander (*Coriandum sativum L.*) against microbial flora isolated from clinical sample.

Organisms	No of Isolates	Average diameter of Zone of inhibition {to the nearest millimeter(mm)}*			
		Aniseed	Black pepper	Coriander	Bay leaf
<i>Escherichia coli</i>	05	12±0.3mm	22±0.1mm	13±0.2mm	11±0.3mm
<i>Klebsiella pneumonia</i>	03	11±0.3mm	12±0.3mm	13±0.2mm	11±0.3mm
<i>Staphylococcus aureus</i>	05	14±0.1mm	16±0.1mm	11±0.3mm	11±0.3mm
<i>Pseudomonas aeruginosa</i>	04	–	–	–	–
<i>Proteus mirabilis</i>	04	12±0.3mm	13±0.2mm	11±0.3mm	13±0.2mm
<i>Bacillus subtilis</i>	04	12±0.3mm	11±0.3mm	12±0.3mm	11±0.3mm
<b>Totals</b>	<b>25</b>				

\*Including the diameter of filter paper Disc-6mm, (-) in parenthesis shows no antibacterial activity/ inhibitory zone.



**Figure 1:** Antimicrobial activity of black pepper and coriander against *Staphylococcus aureus*



**Figure 2:** Antimicrobial activity of used spices against *E.coli* and *Proteus*

A study for antimicrobial potential of extract of spices against clinical isolates was conducted. For this purpose 25 different number of clinical isolates belong to 6 different genera of gram+ve and gram-ve organism were used including *Escherichia coli* (5 isolates), *Klebsiella pneumoniae* (3 isolates), *Staphylococcus aureus* (5 isolates), *Pseudomonas aeruginosa* (4 isolates), *Proteus mirabilis* (4 isolates), and *Bacillus subtilis* (4 isolates). The data representing the antimicrobial activity of spice extract against clinical isolates is present in Table-I-II. The extract of black pepper, aniseed, coriander and bay leaf exhibit antimicrobial activity against all tested microorganism with an highest inhibition zone diameter (INZ) ranging from 22mm to 16mm as shown in Table-II. The inhibition zone diameter was maximum against *Escherichia coli* (22±0.1mm) followed by *Staphylococcus aureus* (16±0.1mm), *Proteus mirabilis* (13±0.3mm), *Klebsiella pneumoniae* (13±0.3mm) and *Bacillus subtilis* (11±0.3 mm). At the end of the analyses, we can say that black pepper, aniseed, coriander and bay leaf exhibits antimicrobial activity against all tested organism except *Pseudomonas*.

## DISCUSSION

Chemical constituents of the spices favors the antibacterial activity and should be valuable in defense. Data from this study helps to control the resistant bacteria which are becoming a threat to human health and this can serve as an important platform for the development of inexpensive, safe and effective natural medicines for the cure of infections and diseases.

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