ORIGINAL ARTICLE

BACTERICIDAL ACTIVITY OF BLACK PEPPER, BAY LEAF, ANISEED AND CORIANDER AGAINST ORAL ISOLATES

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ABSTRACT
Present investigation focused on antibacterial potential of aqueous decoction of black pepper (*Piper nigrum L.*), bay leaf (*Laurus nobilis L.*), aniseed (*Pimpinella anisum L.*), and coriander (*Coriandum sativum L.*) against 176 bacterial isolates belonging to 12 different genera of bacterial population isolated from oral cavity of 200 individuals. The disc diffusion technique was employed. Overall aqueous decoction of black pepper was the most bacterial-toxic exhibited 75% antibacterial activity as compared to aqueous decoction of bay leaf (53.4%) and aqueous decoction of aniseed (18.1%), at the concentration of 10µl/disc. The aqueous decoction of coriander did not show any antibacterial effect against tested bacterial isolates.

Keywords: *Pimpinella anisum L.*, *Coriandum sativum L.*, *Piper nigrum L.*, *Laurus nobilis L.* and aqueous decoction.

INTRODUCTION

Recently there has been a renewed interest in improving health and fitness through the use of more natural products. Herbs and spices are an important part of the human diet. They have been used for thousands of years to enhance the flavor, color and aroma of food. In addition to boosting flavor, herbs and spices are also known for their preservative and medicinal value (deSouza, 2005) which forms one of the oldest sciences. Yet it is only in recent years that modern science has started paying attention to the properties of spices.

Spices can be defined as "any dried, fragrant, aromatic or pungent vegetables or plant substances in whole, broken or ground forms that contribute flavor, whose primary function in food is seasoning rather than nutrition and that may contribute piquancy of foods and beverages". Although as natural substances spices are easily absorbed by our bodies and generally do not have any adverse effects, spices as medicine should be used judiciously. This is because substances' being derived from a plant does not mean it is always harmless. The latest finding suggests that the chemicals present in spices can be allergens, carcinogens, and mutagens (Chandarana *et al*., 2005). Keeping this view, the present study was conducted to determine the antibacterial potential of aqueous decoction of aniseed (*Pimpinella anisum L.*), coriander (*Coriandum sativum*), black pepper (*Piper nigrum L.*), and bay leaf (*Laurus nobilis L.*) against oral isolates. Aniseed (*Pimpinella anisum L.*) is the schizocarpic fruit of the annual anise plant of the parsley family (Umbelliferae) and comes from the Middle East and has become native to the countries of the Mediterranean. Aniseed is one of the oldest spices and is also used medicinally. It contains 1.5-4% volatile oil (about 80% antheole) and other ingredients, which have a strong seasoning action including coumarins, glycosides, fixed oils, 30% fatty oils, choline (Mills, 1993). The volatile oil in aniseed provides the basis for its internal use to ease griping, intestinal colic and flatulence. It also has a marked expectorant and antispasmodic action and may be used in bronchitis, in tracheitis where there is persistent irritable coughing, and to reduce the symptoms of whooping cough. Externally, the oil may be used in an ointment base for the treatment of scabies and lice infestations. Aniseed's mild oestrogenic effects, thought to be due to the presence of diantheole and photoantheole, explain the use of this plant in folk medicine to increase milk secretion, facilitate birth and increase libido (Ody, 1993).

Coriander (*Coriandrum sativum L.*, Umbelliferae) is considered both an herb and a spice since both its leaves and seeds are used as a seasoning condiment. The name coriander is derived from the Greek word *koris* which means bug and commonly famous as Cilantro. It is native to the Mediterranean and Middle Eastern regions and has been known in Asian countries for thousands of years. Coriander seeds have a health-supporting reputation that is high on the list of the healing spices. It has traditionally been referred to as an anti-diabetic (Gray and Flatt, 1999), anti-inflammatory and recently been studied for its cholesterol-lowering effects (Chithra and Leelamma, 1999). In addition, it is also used as carminative, diuretic, tonic, stimulant, stomachic, refrigerent, aphrodisiac and analgesic. The coriander seeds contain 0.5-1% essential oil and are rich in beneficial phytonutrients including carvone, geraniol, limonene, borneol, camphor, elemol, and linalool. Coriander's flavonoids include quercitin, kaempferol, rhamnetin, and epigenin. Coriander also contains active phenolic acid compounds including caffeic and chlorogenic acid.

Research also suggests that the volatile oils found in the leaves of the coriander plant may have antimicrobial properties against food born pathogen, such as Salmonella species (Kubo *et al*., 2004).

Black pepper (*Piper nigrum L.*, Piperaceae) is used to treat asthma, chronic indigestion, colon toxins, obesity, sinus, congestion, fever (Ravindran, 2000), intermittent fever, cold extremities, colic, gastric ailments and diarrhea (Ao *et al*., 1998). Black pepper is native to India and has been a prized spice since ancient times. It has been shown to have antimicrobial activity (Dorman & Deans, 2000). Both aqueous and ethanolic extracts of black pepper have been screened for antibacterial activity against a penicillin G resistant strain of *Staphylococcus aureus* (Perez and Anesini, 1994), *Bacillus cereus* and *B. subtilis* (Singh *et al*., 2005). Piperine ([1-[5-[1,3-benzodioxol-5-yl]-1-oxo-2, 4, pentadienyl piperidine), a pungent alkaloid present in black pepper, enhances the bioavailability of various structurally and therapeutically diverse drugs. A concise mechanism of its bioavailability enhancing action is poorly understood. However, data suggests that piperine is absorbed very fast across the intestinal barrier; it may form non-polar complexes with drugs and solutes thus increasing permeability across the barriers (Khajuria *et al*., 2002). Platel *et al*. (2003) showed that the spice mix of coriander, turmeric, red chili, black pepper and cumin favorably enhance the pancreatic lipase, chymotrypsin and amylase activity when consumed via diet. In addition, these spice mix brought about a pronounced stimulation of bile flow and bile acid secretion.

Bay leaf (*Laurus nobilis L*., Lauraceae) is culinary plant and has a long tradition of use in the Chinese and Ayurvedic systems of medicine. Bay leaf is traditionally used as an analgesic to treat a variety of complaints, neuralgia and intestinal cramps and still occasionally being valued for its beneficial effect upon the digestive system. Bay leaf oil tested for its bactericidal activity has shown to be active against *Salmonella enterica* and *E. coli* (Friedman *et al*., 2002). Ethanol, water and n-Hexane extracts of bay leaves have been evaluated for cytotoxic properties using the brine shrimp bioassay. This study indicates that only the n-hexane extract exhibites cytotoxic activity (Kivcak and Mert, 2002). The microbial growth inhibitory properties of an essential oil of *Laurus nobilis* were studied by the determination of the minimum inhibitory concentration (MIC) against bacteria, fungi and yeasts. The essential oils extracted from bay leaf had antimicrobial activity (Raharivelomanana *et al*., 1989). Essential oil of bay leaves is extremely useful in preventing migraine, absorption of insulin in the body and treatment of skin problems. Besides, the aqueous infusion obtained from the bay leaves have been used for many years in European women to ease the pains of afterbirth while aqueous decoction as a wash on sores and to remove vermin from the head.

MATERIALS AND METHODS

Bacterial strains

A total of 176 bacterial isolates belonging to 12 different genera of bacterial population were isolated from oral cavity of 200 individuals of both sexes (Male and Female), aged between 2-85 years. These individuals belonged to different localities of Karachi region. Distribution of isolates is presented in table 1. All isolates were characterized to specie level according to standard diagnostic criteria as described by Sonnenwirth and Jarett, (1980), Sneath, (1986) and Facklam, (2002).

Table 1: Distribution of bacterial isolates from oral cavity

Organisms	Number of isolates	%
Aeromonas hydrophila	2	1.1
Alcaligenes sp.	4	2.2
Citrobacter sp.	3	2.0
Enterobacter aerogenes	2	1.1
Escherichia coli	25	14.2
Flavobacterium sp.	8	4.0
Klebsiella ozaenae	16	9.0
Klebsiella pneumoniae	9	5.1
Micrococcus roseus	3	1.7
Plesiomonas shigelloides	3	1.7
Pseudomonas aeruginosa	19	11.0
Salmonella typhi	5	3.0
Staphylococcus aureus	2	1.1
Streptococcus anginosus	11	6.2
Streptococcus intermedius	7	4.0
Streptococcus mitis	4	2.2
Streptococcus morbillorium	8	4.5
Streptococcus mutans	10	6.0
Streptococcus oralis	7	4.0
Streptococcus salivarius	9	5.1
Streptococcus sanguis	10	5.6
Streptococcus uberis	9	5.1
Total	176	100

Selection of medicinal spices

Four medicinal spices; aniseed (*Pimpinella anisum L.*), coriander (*Coriandum sativum*), black pepper (*Piper nigrum L.*), and bay leaf (*Laurus nobilis L.*); were collected locally. These spices have previously been reported to have antibacterial activity against different bacterial strains (Bonjar, 2004; Ates and Erdogrul, 2003; Perez and Anesini, 1994).

Preparation of aqueous decoction

Aqueous decoctions of aniseed, coriander seed, black pepper seed and bay leaf were prepared by boiling 10g in 100ml sterile distilled water over low flame for 15minutes. The flasks were then plugged and removed from heat and allowed to cool. After cooling the contents of flasks were filtered.

In vitro antibacterial susceptibility studies

Disc diffusion method (Brooks *et al*., 2002) was employed for antibacterial susceptibility assay. Hundred sterilized discs of filter paper (6mm in diameter) were soaked in 1ml of aqueous decoctions of aniseed, coriander seed, black pepper seed and bay leaf for 1-2minutes and then used for screening. The concentration for each disc was 10µl (100discs/ml of aqueous decoction of each spices). Mueller-Hinton agar (MHA) (Merck) was used as antimicrobial susceptibility test medium and Mueller-Hinton broth (MHB) (Merck) was used for preparation of inoculum. A sterile inoculating loop was touched to four-five isolated colonies of the test bacterial strains grown on MHA and used to inoculate a tube of Mueller-Hinton broth. The inoculated tube was incubated at 37°C for 24hours and standardized to match with 0.5 McFarland turbidity standard. A sterile cotton swab was dipped into the standardized bacterial test suspension and used to evenly inoculate the entire surface of MHA plates. Previously soaked discs in aqueous decoctions of aniseed, coriander seed, black pepper seed and bay leaf were placed on the surface of inoculated plates with sterile forcep. All plates were incubated at 37°C for 24hours. The diameters of the zones of inhibition appearing around the discs were measured to the nearest millimeter (mm) and recorded.

RESULTS AND DISCUSSION

One hundred and seventy six bacterial isolates belonging to 12 different genera of gram positive and gram negative organisms were used in the present study. *In vitro* antibacterial activity results of the aqueous decoction of black pepper, bay leaf, aniseed, and coriander are given in table 2 and fig. 1. The diameter of inhibitory zones recorded includes the size of filter paper disc (6 mm in diameter) and concentration of decoctions of selected spices for each disc was 10µl.

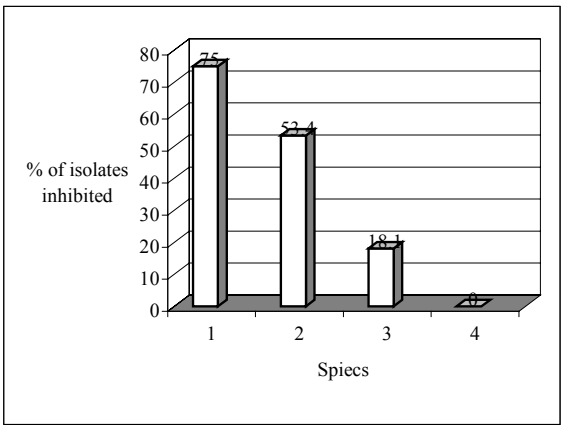

Fig. 1: Comparison of antibacterial activities of aqueous decoctions of selected spices against oral isolates.
Key: 1-Black pepper, 2-Bay leaf, 3-Aniseed, 4-Coriander

Present study indicated great variation in antimicrobial activities of aqueous decoctions against selected spices (fig. 1). The most promising spice was black pepper. The results showed that the aqueous decoction of black pepper possesses great antibacterial potential (75%) as compared to aqueous decoction of bay leaf (53.4%) and aniseed (18.1%) while aqueous decoction of coriander did not show any antibacterial effects.

In the present study, the aqueous decoction of black pepper exhibited maximum effect against *Staphylococcus aureus* (23mm average diameter of inhibitory zone) and found to be most active antibacterial agent against all bacterial isolates except *Salmonella typhi*, *Streptococcus intermedius*, *Streptococcus mitis*, *Streptococcus salivarius*, *Streptococcus sanguis* and *Streptococcus uberis* (table 2).

Findings of the present study are similar to those reported by Perez and Anesini, (1994). They observed the anti-microbial effect of black pepper and found remarkable inhibition against variety of tested bacteria including penicillin G resistant strain of *Staphylococcus aureus*. In another study, inhibition of the growth of meat spoilage bacteria has been reported (Outtara et al., 1997). The major constituent of black pepper is Piperine. It is bioactive compound and has been reported to be the major contributors to the antimicrobial activity of spices.

In the present research, the antibacterial effect of aqueous decoction of bay leaf was next to black pepper. It exhibited maximum antibacterial potential against *Micrococcus roseus* (21mm). Of the four tested spices, only aqueous decoction of bay leaf inhibited the growth of *Salmonella typhi*. The results of present investigation are in correlation with the study in which extracts and essential oil of bay leaf completely inhibited the growth of *Salmonella enterica* and *E. coli*. (Friedman *et al*., 2002). Another study was carried out by Aktug and Karapinar (1986). They investigated antibacterial effect of ground bay leaf and their extracts on *Staphylococcus aureus*, *Salmonella typhimurium* and *Vibrio parahaemolyticus* but did not find any good bactericidal effects.

Another spice used in the present study was aniseed. The aqueous decoction of aniseed exhibited maximum antibacterial activities against *Micrococcus roseus* (15mm). The results of present study are in harmony with the study carried out by Elgayyar *et al*. (2001) in which aniseed has exhibited broad antibacterial spectrum against Gram positive and Gram negative bacteria including *Pseudomonas aeruginosa*, Staphylococcus aureus and *Yersinia enterocolitica*.

In the present study, antibacterial effect of aqueous decoction of coriander was also evaluated. The aqueous decoction of coriander did not possess any antibacterial

Table 2: Antibacterial activities of aqueous decoction 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 human oral cavity

Organisms	*No. of isolates*	*Average diameter of zone of inhibition** {to the nearest millimeter (mm)}			
		Black pepper	*Bay leaf*	*Aniseed*	*Coriander*
Aeromonas hydrophila	2	10	8	11	0
Alcaligenes sp.	4	8	0	11	0
Citrobacter sp.	3	14	0	6	0
Enterobacter aerogenes	2	8	0	0	0
Escherichia coli	25	10	12	0	0
Flavobacterium sp.	8	12	14	0	0
Klebsiella ozaenae	*16*	10	18	0	0
Klebsiella pneumoniae	9	14	7	0	0
Micrococcus roseus	3	15	21	15	0
Plesiomonas shigelloides	3	21	0	0	0
Pseudomonas aeruginosa	19	20	11	0	0
Salmonella typhi	5	0	10	0	0
Staphylococcus aureus	2	23	0	0	0
Streptococcus anginosus	11	10	0	0	0
Streptococcus intermedius	7	0	14	11	0
Streptococcus mitis	4	0	0	12	0
Streptococcus morbillorium	8	13	0	0	0
Streptococcus mutans	10	14	0	0	0
Streptococcus oralis	7	10	0	0	0
Streptococcus salivarius	9	0	0	14	0
Streptococcus sanguis	10	0	0	0	0
Streptococcus uberis	9	0	0	0	0

***** Including the diameter of filter paper Disc-6mm, (0): zero in parenthesis shows no antibacterial activity/ inhibitory zone

potential. In contrary, some workers have found that coriander has strong antibacterial activity against *S. aureus*, *S. typhi* and *E. coli* (Al-Jedah *et al*., 2000).

In general, the mechanisms by which microorganisms survive the action of antimicrobial agents are poorly understood and remain debatable. On the other hand, the chemical constituents of these extracts may have a causal role *in vivo* prevention of diseases caused by bacteria, fungi and yeast. Nevertheless, at least in part if not all, they should be valuable in the multi-chemical defens against microbial attack.

It is obvious that present study has revealed the importance of spices to control resistant bacteria which are becoming a threat to human health. This scientific information can serve as an important platform for the development of inexpensive, safe and effective natural medicines.

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