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IN VITRO ANTIBACTERIAL ACTIVITIES OF KALONJI, CUMIN AND POPPY SEED

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Abstract

Antibacterial activity of aqueous infusions and aqueous decoctions of kalonji (*Nigella sativa* L., Ranunculaceae), cumin (*Cuminum cyminum* L., Umbelliferae) and poppy seed (*Papaver somniferum* L., Papaveraceae) were investigated against 188 bacterial isolates belonging to 11 different genera of Gram +ve and Gram -ve microorganisms isolated from oral cavity of apparently healthy individuals. Disc diffusion method was performed to test antibacterial activity. The highest antibacterial potential was observed from the aqueous decoction of cumin which inhibited 73% of the tested microorganisms than aqueous decoctions of kalonji (51%) and poppy seed (14.4%). In case of tested aqueous infusions, kalonji and cumin showed inhibitory potential against 17% and 5.9% tested microorganisms, respectively. Besides, all isolates were found resistant to aqueous infusion of poppy seed.

Introduction

The spread of multi-drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages, spices have evoked interest as sources of natural products for their potential uses as alternative remedies to heal many infectious diseases (Parekh *et al*., 2005). Spices are the common dietary adjuncts that contribute to the taste and flavor of foods as well as are recognized to stabilize the foods from the microbial deterioration (Kizil & Sogut, 2003). Several scientific reports have described the inhibitory effect of spices on a variety of microorganisms, although considerable variation for resistance of different microorganisms to a given spice and of the same microorganisms to different spices has been observed (Arora & Kaur, 1999). Spices are rich source of biologically active antimicrobial compounds. The Gram +ve bacterial strains are more sensitive to the antimicrobial compound of spices than Gram -ve (Lia & Roy, 2004; Russel, 1991). The extent of antimicrobial activity of spices depend on several factors which includes: 1) kind of spice, 2) composition and concentration of spice, 3) microbial species and its occurrence level, 4) substrate composition and, 5) processing conditions and storage (Shelef, 1983). There is extensive scientific literature on the antimicrobial potential of spices which have been reviewed by several research scientists (Lanciotti *et al*., 2004; Sagdic *et al*., 2003). The present study gives an access on the antibacterial effects of aqueous infusions and aqueous decoctions of cumin, kalonji and poppy seeds against oral flora.

Materials and Method

Test organisms: A total of 188 isolates belonging to 11 different genera of Gram +ve and Gram -ve bacteria isolated from oral cavity of apparently healthy individuals were used for the study. All isolates were characterized to species level according to standard diagnostic criteria as described by Sonnenwirth & Jarett (1980), Baron *et al*., (1994) and Facklam (2002). The isolates were maintained on Tryptic Soy Agar or Nutrient Agar medium.

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Collection of spices: All samples of spices viz., kalonji (*Nigella sativa* L.), cumin (*Cuminum cyminum* L.) and poppy seed (*Papaver somniferum* L.) were purchased from the local market of Karachi, Pakistan.

Preparation of aqueous infusion: Aqueous infusions of cumin, kalonji and poppy seed were prepared by steeping 10g in 100ml sterile distilled water in sterile flasks separately. The flasks were kept for 48h at room temperature with occasional shaking. The contents of flasks were filtered.

Preparation of aqueous decoction: Aqueous decoctions of cumin, kalonji and poppy seed were prepared by boiling 10g in 100ml sterile distilled water over low flame for 15 minutes. The flasks were then plugged and removed from heat and allowed to cool. After cooling the contents of flasks were filtered.

Determination of antibacterial activity: Screening of antibacterial activity was performed using disc diffusion method (Brooks *et al*., 2002).

Base medium: Tryptic Soy Broth (TSB) and Tryptic Soy Agar medium (TSA) were used for the determination of antibacterial activities of cumin, kalonji and poppy seed.

Preparation and standardization of inoculum: A sterile inoculating loop was touched to 4-5 isolated colonies of the bacterial species grown on agar and then used to inoculate a tube of TSB. The inoculated TSB tube was incubated for 24h at 35-37^{o}C and was matched to 0.5 McFarland turbidity standard.

Disc diffusion assay: Hundred sterilized filter paper discs of 6mm diameter were soaked in 1ml of aqueous infusions or aqueous decoctions of cumin, kalonji and poppy seed for 1-2 minutes. Thus, potency of each disc was 10µl. The soaked discs were used for screening. A sterile cotton swab was dipped into the standardized bacterial test suspension and used to evenly inoculate the entire surface of a TSA plate. Five minutes were allowed to dry and previously soaked discs with aqueous infusions and decoctions of cumin, kalonji and poppy seeds were placed on it with sterile forceps. All plates were incubated at 35-37^{o}C for 24h. After incubation, the diameter of zone of inhibition was measured to nearest millimeter (mm).

Statistical analysis: The results were calculated as mean diameter of zone of inhibition in mm ± standard deviation (mean ± SD).

Results and Discussion

Spices are frequently used as an active ingredient in certain medicines and reported to possess a number of pharmacological effects to treat different human ailments (Bonjar *et al*., 2004). Several investigations have been directed towards their antibacterial properties (Voravuthikunchai *et al*., 2005). The present study gives an account on the antibacterial activities of aqueous infusions and aqueous decoctions of some spices viz., cumin, kalonji and poppy seed.

The results pertaining to the antibacterial potential of the tested spices are given in Tables 1 & 2 and Fig. 1 & 2. Among the spices screened, the aqueous decoction of cumin showed highest antibacterial potential against tested bacteria. Aqueous decoction of cumin exhibited promising antibacterial activity against 73% tested bacteria than aqueous decoctions of kalonji (51%) and poppy seed (14.4%) (Fig. 2). Aqueous decoction of cumin was most active against Gram-ve than Gram+ve bacterial species (Table 2). The largest inhibitory zone was found against *Escherichia coli* (23.8mm ± 1.2SD). Previous research studies have documented that *E. coli* are known to be multi-drug resistant (Saeed *et al*., 2007; Singh *et al*., 2002). Present study is in agreement with previous study of De *et al*., (1999) who reported antibacterial potential of different type of spices including cumin and found potent antibacterial potential against *Bacillus subtilis*, *Saccharomyces cerevisiae* and *E. coli*. The aqueous decoction of cumin also exhibited significant inhibitory activity against *Micrococcus roseus* (20.8mm ± 2.3SD), *Plesiomonas shigelloides* (18.5mm ± 8.3SD), *Alcaligenes* spp., (17.1mm ± 2.9SD), *Citrobacter* spp., (16.2mm ± 0.5SD), *Klebsiella pneumoniae* (15.9mm ± 0.8SD), *Aeromonas hydrophila* (15.8mm ± 1.3SD), *Klebsiella ozaenae* (15.2mm ± 1.3SD), *Pseudomonas aeruginosa* (12.3mm ± 3.3SD), *Enterobacter aerogenes* (12.0mm ± 0.1SD) and *Staphylococcus aureus* (8.9mm ± 5.6SD). The principle active constituents of cumin are cuminaldehyde (4-isopropylbenzaldehyde), alpha and beta pinene, aldehyde of perilla, alcohol of cumin, dipentene, para-cymene, beta-phellandrene and its essential oil (Nanasombat & Lohasupthawee, 2005).

In the present study, the antibacterial activity of aqueous decoction of kalonji was found next to cumin. The aqueous decoction of kalonji revealed significant antibacterial potential against *Staphylococcus aureus* (19.6mm ± 1.8SD), *Micrococcus roseus* (17.9mm ± 2.5SD), *Streptococcus mutans* (16.9mm ± 3.9SD), *Streptococcus morbillorium* (16.5mm ± 4.9SD), *Streptococcus sanguis* (14.6mm ± 2.4SD), *Streptococcus intermedius* (13.6mm ± 1.5SD), *Klebsiella ozaenae* (12.8mm ± 3.3SD), *Aeromonas hydrophila* (8.8mm ± 1.5SD), and *Streptococcus salivarius* (8.5mm ± 0.8SD) (Table 2). This study is correlated with the study carried out by Mashhadian & Rakhshandeh (2005) in which kalonji has been reported to inhibit *Staphylococcus aureus*, *Ps. aeruginosa* and *Candida albicans*. It is interesting to note that G+ve bacterial isolates were more sensitive to aqueous decoction of kalonji than G-ve (Salman *et al*., 2005). Present study is also in agreement with Morsi (2000) who reported that kalonji extracts produce antibacterial activity against a broad range of microbes and especially multiple antibiotic resistant bacteria. Preliminary clinical trials have documented its therapeutic use for the treatment of variety of diseases and conditions that include diarrhoea, asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness, influenza and dental caries (Ali & Blunden, 2003; Gilani *et al*., 2001). In addition, different pharmacological effects such as insulinotropic, hypoglycemic, anti-cancer, antinociceptive, antiinflammatory, hepatoprotective, neuroprotective, antihistamine, antiulcer and bronchodilator activities have been reported for kalonji (Hosseinzadeh *et al*., 2007). The seeds of kalonji have over one hundred different chemical constituents including active ingredient thymoquinone (TQ), which is responsible for antibacterial activity (Ali & Blunden, 2003). Other chemical constituents are dithymoquinone, thymohydroquinone, nigellone, ascorbic acid (vitamin C), tocopherol (vitamin E), linoeic acid, lipase, oleic acid, carvacrol, t-anethole and 4-terpineol etc. Kalonji seeds are also source of Ca, K, Fe, Zn, Mg, Se and Na, required only in small amount by the body (Tawab & Fatima, 2006). In the present study, the antibacterial activity of aqueous decoction of poppy seed was also investigated. As compared to antibacterial activities of cumin and kalonji, the aqueous decoction of poppy seed exhibited weak antibacterial activity against *Escherichia coli* (9.5mm ± 0.5SD), *Citrobacter* spp., (8.8mm ± 2.3SD), *Alcaligenes* spp., (8.5mm ± 1.1SD) and *Micrococcus roseus* (8.1mm ± 3.4SD) (Table 2).

Table 1. Antibacterial activities of aqueous infusions of Kalonji, Cumin and Poppy seed.

Organisms	No. of isolates	Mean diameter of zone of inhibition (mm ± SD**)		
		Kalonji	Cumin	Poppy seed
Gram negative bacteria				
Aeromonas hydrophila	2	0	0	0*
Alcaligenes spp.	4	0	8.4 ± 0.6	0
Citrobacter spp.	3	8.1 ± 1.4	11.1 ± 2.1	0
Enterobacter aerogenes	2	0	0	0
Escherichia coli	25	0	13.8 ± 8.4	0
Flavobacterium spp.	8	0	0	0
Klebsiella ozaenae	16	10.2 ± 1.5	8.0 ± 2.2	0
Klebsiella pneumoniae	9	0	0	0
Pseudomonas aeruginosa	19	0	0	0
Plesiomonas shigelloides	3	0	15.5 ± 2.0	0
Gram positive bacteria				
Micrococcus roseus	3	11.6 ± 1.4	8.1 ± 0.7	0
Staphylococcus aureus	2	0	0	0
Streptococcus anginosus	11	0	0	0
Streptococcus intermedius	10	16.1 ± 1.4	0	0
Streptococcus mitis	14	0	0	0
Streptococcus morbillorium	8	0	0	0
Streptococcus mutans	10	0	0	0
Streptococcus oralis	10	0	0	0
Streptococcus salivarius	19	0	0	0
Streptococcus sanguis	10	10.2 ± 1.3	0	0
Total	**188**			

*Zero (0) shows absences of inhibitory zone; **Standard deviation

Table 2. Antibacterial activities of aqueous decoctions of Kalonji, Cumin and Poppy seed.

Organisms	No. of isolates	Mean diameter of zone of inhibition (mm ± SD**)		
		Kalonji	Cumin	Poppy seed
Gram negative bacteria				
Aeromonas hydrophila	2	8.8 ± 1.5	15.8 ± 1.3	0*
Alcaligenes spp.	4	0	17.1 ± 2.9	8.5 ± 1.1
Citrobacter spp.	3	0	16.2 ± 0.5	8.8 ± 2.3
Enterobacter aerogenes	2	0	12.0 ± 0.1	0
Escherichia coli	25	0	23.8 ± 1.2	9.5 ± 0.5
Flavobacterium spp.	8	0	0	0
Klebsiella ozaenae	16	12.8 ± 3.3	15.2 ± 1.3	0
Klebsiella pneumoniae	9	0	15.9 ± 0.8	0
Pseudomonas aeruginosa	19	0	12.3 ± 3.3	0
Plesiomonas shigelloides	3	0	18.5 ± 8.3	0
Gram positive bacteria				
Micrococcus roseus	3	17.9 ± 2.5	20.8 ± 2.3	8.1 ± 3.4
Staphylococcus aureus	2	19.6 ± 1.8	8.9 ± 5.6	0
Streptococcus anginosus	11	0	0	0
Streptococcus intermedius	10	13.6 ± 1.5	0	0
Streptococcus mitis	14	0	0	0
Streptococcus morbillorium	8	16.5 ± 4.9	0	0
Streptococcus mutans	10	16.9 ± 3.9	0	0
Streptococcus oralis	10	0	0	0
Streptococcus salivarius	19	8.5 ± 0.8	0	0
Streptococcus sanguis	10	14.6 ± 2.4	0	0
Total	**188**			

*Zero (0) shows absences of inhibitory zone; **Standard deviation

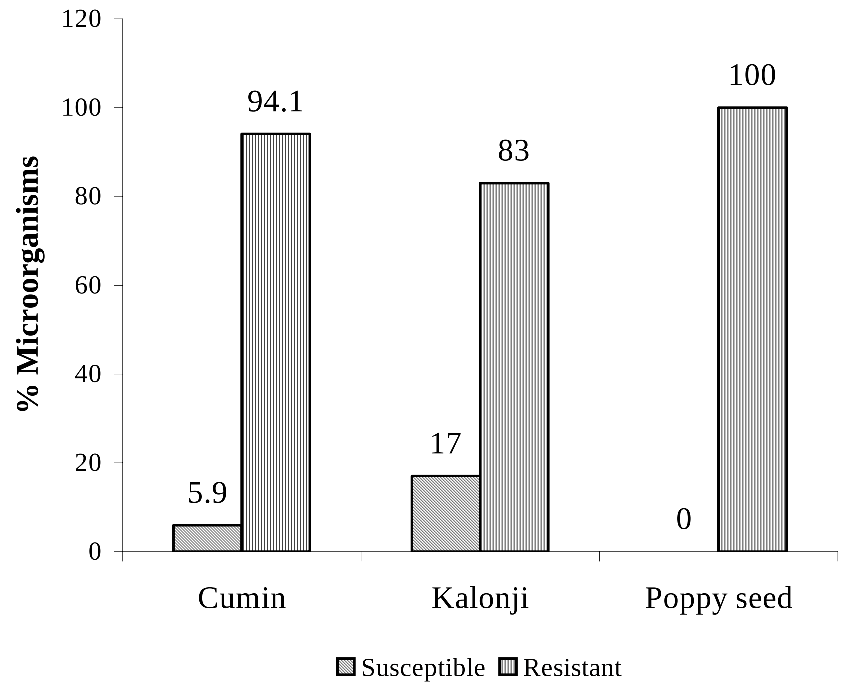

Fig. 1. Comparison of antibacterial activities of aqueous infusions of cumin, kalonji and poppy seed against oral flora.

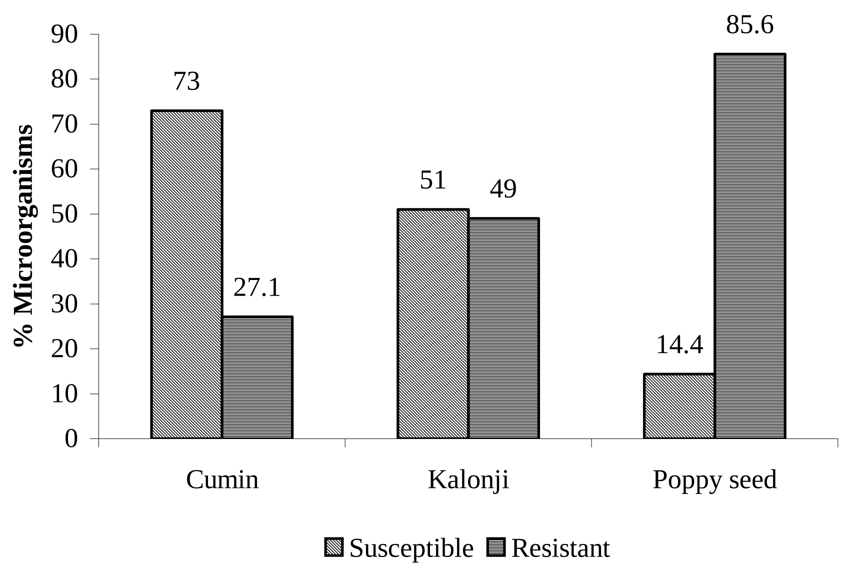

Fig. 2. Comparison of antibacterial activities of aqueous decoctions of cumin, kalonji and poppy seed against oral flora.

Besides, the aqueous infusions of cumin, kalonji and poppy seed were also investigated for the assessment of their antibacterial potential. Most of the bacterial species were found resistant to aqueous infusions of spices (Table 1). However, aqueous infusions of kalonji and cumin exhibited weak antibacterial activity against 17% and 5.9% tested bacteria respectively (Fig. 1). The significant inhibitory activity of aqueous infusion of kalonji was found against *Streptococcus intermedius* (16.1mm ± 1.4SD) followed by *Micrococcus roseus* (11.6mm ± 1.4SD), *Klebsiella ozaenae* (10.2mm ± 1.5SD), *Streptococcus sanguis* (10.2mm ± 1.3SD), and *Citrobacter* spp., (8.1mm ± 1.4SD) while aqueous infusion of cumin showed inhibitory activity against *Plesiomonas shigelloides* (15.5mm ± 2.0SD), *Escherichia coli* (13.8mm ± 8.4SD), *Citrobacter* spp., (11.1mm ± 2.1SD), *Alcaligenes* spp., (8.4mm ± 0.6SD), *Micrococcus roseus* (8.1mm ± 0.7SD), and *Klebsiella ozaenae* (8.0mm ± 2.2SD) (Table 1). On the other hand, aqueous infusion of poppy seed failed to inhibit any of the tested bacteria (Table 1). The study provides support to the antibacterial potential of cumin and kalonji as a potent antibacterial agent.

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