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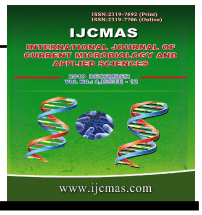

Original Research Article

Antimicrobial activity of bee honey, black cumin oil and green tea against multi-drug resistant pathogenic bacteria

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A B S T R A C T

Four different concentrations (10%, 30%, 50% and 100%) of green tea, honey and black cumin oil were used as antibacterial agents against four multi-drug resistant pathogenic strains.Strains were obtained from Asidairy hospital, Sakaka city, Saudia Arabia. These strains include: *Enterobacter cloacae, Serratia marcescens, Escherichia coli* and *Acinetobacter baumannii.* Fixed volumes (1 ml) of the green tea, honey and black cumin oil poured into wells made in Mueller Hinton agar previously inoculated with a single bacterium. Zones of inhibition were measured after 48h incubation for each bacterium. Results indicated that *E.coli* was the only strain found to be sensitive to green tea (8-13mm).The four strains were sensitive or extremely sensitive to honey at concentrations 50% and 100 % and sensitive or extremely sensitive to black cumin oil at concentrations 30%, 50% and 100%.

Keywords

Antimicrobial; multi-drug-resistant; *Serratia marcescens*; *Escherichia coli; Acinetobacter baumanni;*

Introduction

The evolution and spread of antibiotic resistance, as well as the evolution of new strains of disease causing agents, is of great concern to the global health community. Our ability to effectively treat disease is dependent on the development of new pharmaceuticals, and one potential source of novel drugs is traditional medicine (Frey and Ryan, 2010). The use of traditional medicine to treat infection has been practiced since the origin of mankind.

Green tea is non-fermented tea. The tea is an infusion of leaves that has been consumed for centuries as a beverage and is valued for its medicinal properties. The phytochemical screening of tea revealed the presence of alkaloids, saponins, tannins, catechin and polyphenols (Mbata,2006). Tea leaves are known for its antimicrobial activity against many microorganisms. The primary difference between green tea and black tea is in the fermentation process required to produce tea. In case of black tea the leaves and buds are fermented or oxidized after they have been dried. In green tea the leaves are steamed after they are dried. The antibacterial effect of honeys has been

practiced since along centuries ago and honey is one of the oldest traditional medicines considered to be important in the treatment of several human diseases (Manisha and Shyamapada, 2011). *Nigella sativa* L. (Family *Ranunculaceae)* has been used for centuries in the Middle East, Eastern Europe, Asia and Africa as a natural remedy for many ailments (El-Kadi and Kandil, 1986, Zaher, 2008). In Muslim countries *N. sativa* seeds have been used extensively in folk medicine for treatment of different diseases, as Prophet Muhammad of Islam, stated that the black seed can heal all diseases except for death (Bhatti and Rehman, 2009). *Enterobacter cloacae, Serratia marcescens* and *Escherichia coli* are the common pathogens of human infection.*Enterobacter cloacae* infections are seen commonly in burn victims, immunocompromised patients, and patients with malignancy (Frey and Ryan, 2010). *Serratia marcescens* and *Escherichia coli,* both with a ubiquitous presence in our environments, can be pathogenic in some cases (Singleton, 1999). *Acinetobacter baumannii* has emerged as an importantnosocomial pathogen (Manikal *et al*,2000, Go et al,1994). These bacteria and many others that may be pathogenic are sensitive to antibiotics but rapidly changing and acquiring drug resistance due to overuse of antibiotics in humans (Johnson *et al*,2006).

The present study is designed to assess antibacterial activity of green tea, honey and black cumin oil against multi-drug resistant pathogenic bacteria

Materials and Methods

Preparation of solutions

Honey dilutions were prepared immediately before testing by diluting honey to the required concentrations (10, 30, 50,100% v/v).10g of green tea powder have been soaking for 24 hours using 100ml of distilled water in a 250ml sterile conical flask. The extracts were filtered using Whatman filter paper No 1. The oil of black cumin seeds was diluted using ethylene glycol. Four different concentrations 10, 30, 50 and 100%from tea and oil were used.

Microorganisms used

The pathogens used in this experiment were obtained from Al Amir Asid diary hospital, Sakaka city, Saudia Arabia. Four multi-drug resistant strains include *Enterobacter cloacae, Serratia marcescens, Escherichia coli*and *Acinetobacter baumannii* were used.

Microbiological techniques

The antibacterial studies were conducted according standard method (Lis-Balchin *et al*, 1996c). Four wells of about 0.6 mm diameter were aseptically cut on agar-plate using a sterile cork borer allowing at least 30 mm between adjacent wells and between peripheral wells and the edge of the petri dish. Fixed volumes (1 ml) of the green tea, honey and black cumin oil introduced into wells made in Mueller Hinton agar previously inoculated with a single bacterium. Zones of inhibition were measured after 48hincubation in the dark, at the appropriate temperature for each bacterium.

Results and Discussion

The results of the three antibacterial agents with four concentrations (10, 30, 50, 100% v/v) used in this study are shown in Table1 and figures (1, 2, 3).The antibacterial activity was classified as: no sensitive, for

Table.1 Zone of inhibition in (mm) for the different antimicrobial agents against various multi-drug resistant (MDR) bacteria

MDR strains	Size of zones of inhibition (mm) for concentrations (%)											
	Black cumin oil				Green tea				honey			
	10%	30%	50%	100%	10%	30%	50%	100%	10%	30%	50%	100%
Escherichia coli	0	13	33	38	0	8	9	13	0	7	9	21
Enterobacter cloacae	0	12	18	20	0	6	7	6	0	0	7	20
Serratia marcescens	0	19	6	15	0	2	6	5	0	8	8	16
Acinetobacter baumannii	3	12	9	22	0	0	3	4	0	0	9	23

MDR: multi-drug resistant

Figure.1 Antibacterial activity (mm) of different concentrations of green tea against multi-drug resistant strains

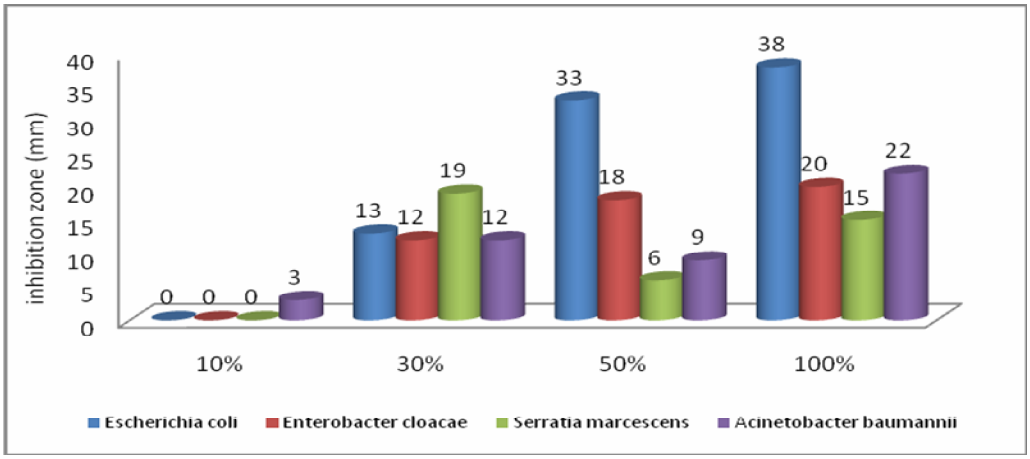


Figure.2 Antibacterial activity (mm) of different concentrations of honey against multi-drug resistant strains

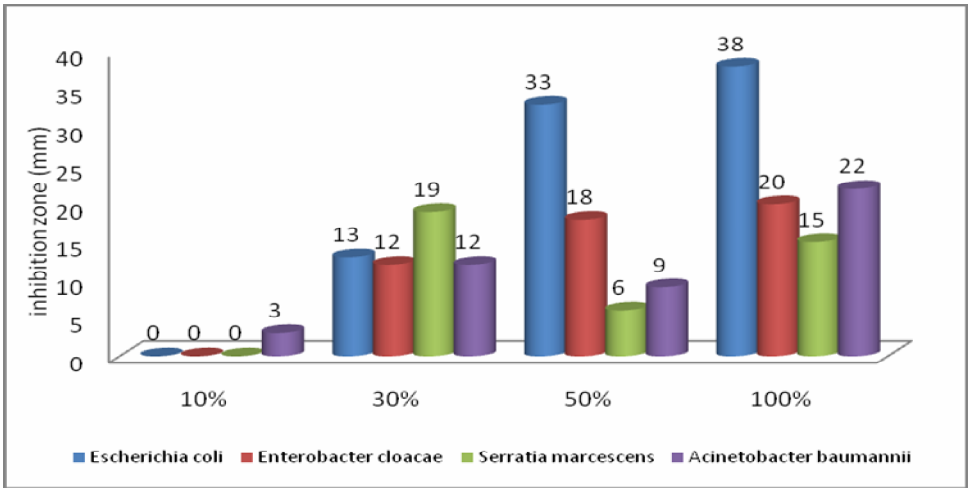
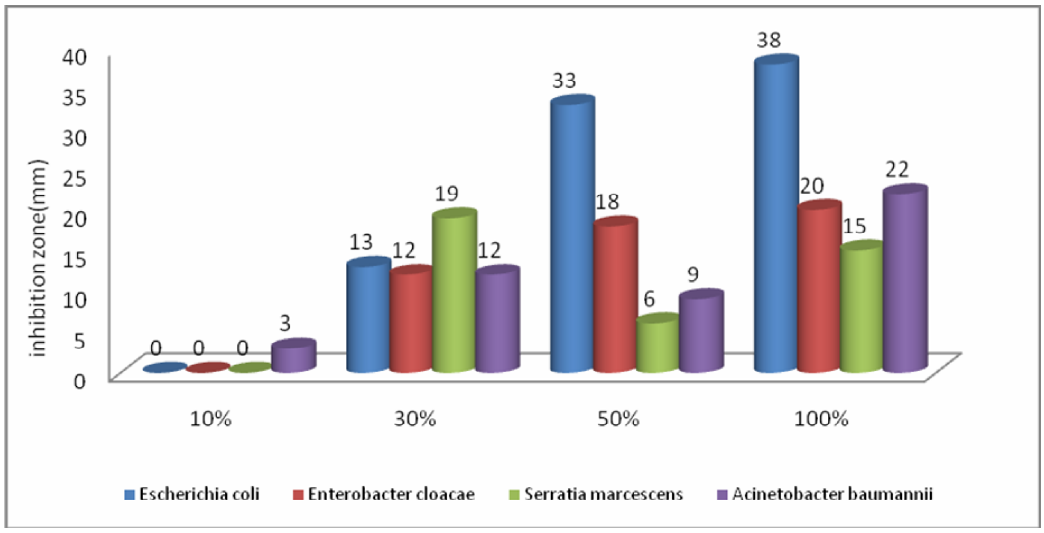


Figure.3 antibacterial activity (mm) of different concentrations of black cumin oil against multi-drug resistant strains



diameters lower than 8 mm; sensitive, for diameters from 8 to 14 mm; very sensitive, for diameters from 15 to 19 mm; extremely sensitive, for diameters higher than 20 mm. In spite of all compounds have been found to possess antibacterial action the results showed variations in their effects. This difference in their activity depends upon the concentration and type of the extracts. These effects may also differ depending on the bacterial species so that they may be either growth inhibitory or stimulatory (Tiwari et al, 2005). *E.coli* multi-drug resistant strain was found to be sensitive to green tea (8-13mm) while the three other strains showed no sensitivity ranged(2-7 mm).These results indicates the presence of potent antibacterial activity, which confirms its use against infection. These observations may be attributed to green teacatechin compounds and polyphenols. These compounds have beenfound to possess antibacterial action (Mbata ,2006).A similar results of Zaher *et al*(2008) andS. Archana and Jayanthi(Archana and Jayanthi,2011) indicated *E.coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Candida albicans* and *Pseudomonas aeruginosa* were sensitive to green tea extracts. Green tea also possesses antimicrobial activity against a variety of pathogenic bacteria that cause cystitis, pyelonephritis, diarrhea, dental caries, pneumonia and skin infection (You,1999).Multi-drug resistant strains of *Enterobacter cloacae*, *Serratia marcescens*, *Escherichia coli* and *Acinetobacter baumannii* were found to be sensitive or extremely sensitive to honey at concentrations 50% and 100 %(Ttable 1 and figure 1, 2, 3). The honey antimicrobial activity against *Escherichia coli* is in agreement of many previous studies. The effect of honey on Gram-negative bacteria was explained by Taormina *et al* (2001) who attributed it to the presence of hydrogen peroxide and powerful antioxidants, as also to a naturally low pH, which is unsuitable for bacterial growth and to the presence of phenolic acids, lysozymeand flavanoids**.**

Chauhan *et al* (2010) reported that *E.coli* and *P.aeruginosa* were susceptible to honey and diameter of inhibition zone

ranged 6.94-35.95 mm. AI-Namma(2009) also observed that honey has a greater inhibitory effect on Gram negative bacteria and*S. typhi*, *P.aeruginosa*, and are more susceptible than other test organisms.

Inhibition zones produced, revealed that black cumin oil have the best antibacterial activity compared to green tea and honey (figure 1, 2 ,3). The results also showed that all strains were sensitive to 30%, 50% and 100% concentrations. The antimicrobial activity of N. sativa crude extracts against different microorganisms has been studied by several research groups. Seed extracts of N. sativa inhibit the growth of *Escherichia coli, Bacillus subtilis* and *Streptococcus fecalis* (Saxena and Vyas, 1986). Morsi (2000) reported the antibacterial activity of crude extracts of N. sativa against multi-drug-resistant organisms, including Gram-positive bacteria like *Staphylococcus aureus* and Gram-negative bacteria like *Pseudomonas aeruginosa* and *Escherichia coli*. *N. sativa* extracts also inhibit the growth of different strains of *H. pylori*(Salem *et al*,2010).It should be mentioned that the antibacterial activity of the tested active constituents of N. sativa was not affected by multi-drug resistance of both clinical gram-positive and Gram-negative bacterial strains. This phenomenon which was previously reported for crude extracts of *N. sativa* (Morsi,2000) suggests that the mechanisms of action of active constituents of *N. sativa* are different to those of antibiotics.

Almost 25 years ago, researchers observed acquired resistance of *A. baumannii* to antimicrobial drugs commonly used at that time, among them aminopenicillins, ureidopenicillins, first and second-generation cephalosporins, cephamycins, most aminoglycosides, chloramphenicol, and tetracyclines (Murray and Moellering, 1979),although *Acinetobacter baumannii*multi-drug resistant was found to be sensitive to black cumin oil and honey.

In conclusion honey and black cumin oil were lethal to four tested bacteria therefore, they might be considered as possible alternatives to antibiotics for treatment of multidrug-resistant strains infections.

In future green need more studies to discover their antimicrobial activity against multi-drug resistant strains studied. It is hoped that this may help to avoid the side effects of antibiotics.

References

AI-Namma, R.T. 2009. Evalution of in vitro inhibitory effect of honey on some microbial isolate. J. Bacteriol. Res. 1(6): 64-67.

Bhatti, I.U., and F.U. Rehman, 2009. Effect of prophetic medicine kalonji (Nigella sativa L.)on lipid profile of human beings: an in vivo approach, World Appl. Sci. J. 6: 1053-1057.

Chauhan, A., V. Pandey, K.M. Chacko and Khandal, R.K. 2010. Antibacterial activity of raw and processed honey. Electronic. J. Biol. 5: 58- 66.

El-Kadi, A., and Kandil,O. 1986. Effect of Nigella sativa (the black seed) on immunity. Bulletin Islamic Med. 4: 344-348.

Frey, F.M., and Ryan, M. 2010.Antibacterial activity of traditional medicinal plants used by Haudenosaunee peoples of New York State. BMC Complem. Altern. Med.10:64.

Go, E.S., C. Urban, J. Burns, B.

Kreiswirth, W. Eisner , Mariano N, et al. 1994. Clinical and molecular epidemiology of Acinetobacter infections sensitive only to polymyxin B and sulbactam. Lancet. North. Am. Ed. 344:1329–32.

Johnson, J.R., M.A. Kuskowski and Menard, M. 2006. Similarity between human and chicken Escherichia coli isolates in relation to ciprofloxacin resistance status. J. Infect. Dis. 194: 71-78.

Lis-Balchin, M., S.G. Deans and Hart, S. 1996c Bioactivity of commercial geranium oil from different sources. J.Essent. Oil. Res. 8: 281-290.

Manikal, V.M., Landman D, Saurina G, Oydna E, Lal H, and Quale J.2000. Endemic carbapenem-resistant Acinetobacter species in Brooklyn, New York: citywide prevalence, interinstitutional spread, and relation to antibiotic usage. Clin. Infect Dis. 31:101–6.

Manisha Deb, M., and Shyamapada, M. 2011. Honey: its medicinal property and antibacterial activity. Asian. Pac. J. Trop. Biomed. 154-160.

Mbata, T.I., L. Debiao and Saikia, A. 2006. Antibacterial Activity Of The Crude Extract Of Chinese Green Tea (Camellia sinensis) On Listeria monocytogenes . The Internet J. Microbiol. 2006: 2(2).

Morsi, N.M., 2000. Antimicrobial effect of crude extracts of Nigella sativa on multiple antibiotic resistant bacteria. Acta Microbiol. Polonica. 49: 63-74.

Murray, B.E., and Moellering, R.C. Jr. 1979. Aminoglycoside-modifying enzymes among clinical isolates of Acinetobacter calcoaceticus subsp. anitratus: explanation for high-level aminoglycoside resistance. Antimicrob Agents Chemother. 15:190–9.

Archana, S., and Jayanthi Abraham. 2011. Comparative analysis of antimicrobial activity of leaf extracts from fresh green tea, commercial green tea and black tea on pathogens. J. Appl. Pharma. Sci. 01 (08): 2011: 149-152.

Salem, E.M., T. Yar, A.O. Bamosa, A. Al-Quorain, M.I. Yasawy, R.M. Alsulaiman and M.A. Randhawa, 2010. Comparative study of NigellaSativa and triple therapy in eradication of *Helicobacter Pylori* in patients with non-ulcer dyspepsia. Saudi J. Gastoentrol. 16: 207-214.

Saxena, A., and K. Vyas, 1986. Antimicrobial activity of seeds of some ethnopharmacinal plants. J. Econ. Taxon. Bot. 8: 291-299.

Singleton, P., 1999. Bacteria in Biology, Biotechnology and Medicine, 5th ed. England. John Wiley & Sons Ltd.

Taormina, P.J., Niemira BA, and Bauchat, L.R. 2001. Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power, Int .J. Food. Microbiol. 69: 217-225.

Tiwari, R.P, Bharti S.K., Kaur H.D., Dikshit R.P. and Hoondal G.S.2005. Synergistic antimicrobial activity of tea and antibiotics. J. Med. Res. 122: 80-84.

You, S., 1999. Study on feasibility of Chinese green tea polyphones for preventing dental caries. 28:197-199.

Zaher, K.S., W.M. Ahmed and S.N. Zerizer, 2008. Field trial of Observations on the Biological Effects of Black Cumin Seed (*Nigella sativa*) and Green Tea (*Camellia sinensis*), Global Vet. 2: 198-204.