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ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 09, Issue, 12, pp.9112-9115, December, 2018

RESEARCH ARTICLE

INDIAN SPICES: A GLOBAL BENEFIT TO HEALTH

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ARTICLE INFO

Article History:
Received 28th September, 2018
Received in revised form
03rd October, 2018
Accepted 11th November, 2018
Published online 20th December, 2018

Key words:
Drinking water,
Escherichia coli, Spices.

ABSTRACT

Medoavrutta Spices have become important due to their potential antimicrobial effects. The present work aimed to find out the antimicrobial activity of natural spices on multi-drug resistant *Escherichia coli* isolated from drinking water. Anti-bacterial potentials of crude extracts Black pepper (*Piper nigrum*), Coriander (*Coriander sativum*), Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) were tested against ten *Escherichia coli* isolated from drinking water sources at Nagpur. Total 28 drinking water samples were analysed, out of which 10 samples were found to be contaminated with *E. coli*. Isolated *E. coli* were tested against 8 different antibiotics. It was found that all isolates of *E. coli* (100%) were found to be resistant to Carbenicillin, Cefuroxime and Amoxyclave while 100% *E. coli* found to be sensitive to Levofloxacin and Gatifloxacin. Total 90% were sensitive to Azithromycin followed by 80% to Doxycycline Hydrochloride and 50% to Cefixime. When *E. coli* isolates were tested against 4 spices such as Black Pepper, Coriander, Garlic and Ginger, it was found that 50% *E. coli* were found to be sensitive to Garlic and Ginger each followed by 40% to Coriander and 30% to Black Pepper.

Citation: Chandekar, C.J. and Pranita A. Gulhane. 2018. "Traceability program digital in the central equipment and sterilization, to improve the practices of quality in a hospital of third level", *Asian Journal of Science and Technology*, 09, (12), 9112-9115.

INTRODUCTION

Spices traditionally have been used as coloring agents, flavoring agents, preservatives, food additives and as well as antiparasitic, antihelmintic, analgesic, expectorant, sedative, antiseptic and anti-diabetic substances in many parts of the world (K.W. Lee *et al*., 2004). In addition, they possess biological activities such as that of antioxidants (K. Miura *et al*., 2002) and hypocholesterolemics (W.J. Craig, 1999). The antimicrobial effectiveness of mustard, clove, cinnamon and their essential oils were reported for the first time around 1880's. Antimicrobial effectiveness of spices depend on the kind of spice, its composition and concentration, type and concentrations of the target microorganism, substrate composition, and processing and food storage conditions. Multi-drug resistant strains of *Escherichia coli* are widely distributed in hospitals and are increasingly being isolated from community (M. Akram *et al*., 2007). *Escherichia coli* (also known as *E. coli*) is a Gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus *Escherichia* (P. Singleton, 1999). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts. Some *E. coli* are pathogenic, meaning they can cause illness, either diarrhea or illness outside of the intestinal tract. The types of *E. coli* that can cause diarrhea can be transmitted through contaminated water or food.

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Thus, it is urgent need to find out new antimicrobial agents. However, new families of antimicrobial agents will have a short life expectancy (A. Coates *et al*., 2002). For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against multidrug resistant microbe strains (L.C. Braga *et al*., 2005). There are some homemade remedies that we can use in order to destroy *E. coli*. There are some such spices which we use in kitchen in our routine life. In this project some spices have been used which were found to inhibit the growth of *E. coli* which were resistant to some of the broad spectrum antibiotics. These spices are to be describing along with their general role and use as follows:

Black pepper (*Piper nigrum*) is in the family Piperaceae. Pepper oil is also used as ayurvedic massage oil and used in certain beauty and herbal treatments. Coriander powder is derived from the seeds of the coriander plant (*Coriander sativum*). Coriander has pain-relieving properties and is useful for headaches, muscle pain, stiffness and arthritis. It is helpful for the digestive tract, and is good for increasing appetite, and relieving nausea, diarrhea, flatulence and indigestion. *Allium sativum*, commonly known as garlic, which was used as an antiseptic to prevent gangrene during World Wars I and II. A meta-analysis of observational epidemiological studies found that garlic consumption is associated with a lower risk of stomach cancer in the Korean population (H.D. Woo *et al*., 2014). One news source reported that garlic supplements may prevent the common cold, but there is insufficient clinical

research to confirm this effect (F. Brinker, 1995). Garlic along with cinnamon is used as a fish and meat preservative (Rakshit and Madhumita, 2012). Ginger (*Zingiber officinale*) is a flowering plant, in the family Zingiberaceae whose rhizome, ginger root or simply ginger, is widely used as a spice or a folk medicine. Ginger also has a role in traditional ayurvedic medicine. It is an ingredient in traditional Indian drinks, both cold and hot, including spiced Masala chai. In this study crude extract of these spices was used against multidrug resistant strains, where modern antibiotic therapy has limited effect. The spices are generally used as food additives in order to provide taste, smell, colour and also exhibited antibacterial activity. The present study aimed to investigate in vitro antibacterial activity of spices include Black pepper (*Piper nigrum*), Coriander (*Coriandrum sativum*), Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) against multidrug resistant *Escherichia coli* isolated from drinking water.

MATERALS AND METHODS

Sample Collection: Total 28 drinking water samples were collected in sterile glass bottles from various sources (Table 2). Out of which 10 drinking water samples were found to be contaminated with *Escherichia coli*.

Isolation and Identification of organism: Drinking water samples were collected from different drinking water sources. The isolated bacteria were identified on the basis of morphological, cultural and biochemical characteristics (J.G. Collee and W. Marr, 1996).

Spices used in the Study: A total of 4 types of spices were used in the study such as Black pepper *(Piper nigrum*), Coriander *(Coriander sativum*), Garlic *(Allium sativum*) and Ginger *(Zingiber officinale*).

Preparation of Spices Extract: For the preparation of spices extract Black Pepper, Coriander seeds, Garlic and Ginger were bought from local market. These were thoroughly washed to remove the dirt and then grounded using a blender. Twenty gram of the ground material (all 4 spices) were soaked in 100 ml of hot sterile distilled water and allowed to stand for 72 hours at room temperature. After 72 hours it was filtered by using muslin cloth and then used it for further process (R.A. Onyeagba *et al*., 2004; S. Rahman *et al*., 2011).

Antibiotic Susceptibility Test: Pure cultures of *E. coli* were tested for antibioitic susceptibility by the disc diffusion method using eight different antibiotics (Table 1). Antibiotic sensitivity test was performed by Kirby Bauer Disc Diffusion method (Bauer *et al*., 1966). *E. coli* strains were grown on nutrient agar at 37^0C for 24 hours and the colonies were suspended in sterile saline water equivalent to a 0.5McFarland standard (1.5X108CFU/ml). Hi-sensitivity agar plate was uniformly seeded by adding 100µl inoculated broth and was spread by means of spreader. The discs were placed on each inoculated Hi-sensitivity agar plate. The plates were incubated at 37^0C for 18 hours. The diameter of the zone of inhibition was observed in mm and the isolates were classified as "resistant" or "sensitive" based on the standard interpretative chart according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2007).

Antibacterial activity of spices against E. coli: Spices were used against *E. coli* isolates by agar well diffusion method. *E. coli* strains were grown overnight on nutrient agar at 37^0C, and the colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard (1.5×108 CFU/ml). The suspension (100 µL) was spread over the Hi- Sensitivity agar. The wells of 6 mm diameter were cut into the agar medium with a sterilized cork borer. Then 20µl each of the extracts were added separately into the separate wells. The plates were incubated at 37^0C for 18 hours. The diameter of the zone of inhibition around each well was measured and recorded (A.W. Bauer *et al*., 1966).

Table 1. Antibiotics Used in the Study

Azithromycin	15mcg	Cefuroxime	30mcg
Amoxyclave	30mcg	Doxycycline Hydrochloride	30mcg
Carbenicilline	100mcg	Gatifloxacin	5mcg
Cefixime	5mcg	Levofloxacin	5mcg

RESULTS

The present study was conducted to evaluate the effect of different spices on drinking water *E. coli*. In the present study total 28 drinking samples were analysed for the presence of *E. coli*, out of which 10 samples (35.71%) were found to be contaminated with *E. coli.* Water cooler samples were (66.66%) contaminated followed by Water Purifier (57.14%) and Tap water (30.76%) with *E. coli* (Table 2). The isolated *E. coli* were tested against 8 different antibiotics (Table 1). It was found that all the isolates of *E. coli* (100%) were found to be resistant to Carbenicillin, Cefuroxime, and Amoxyclave while 100% *E. coli* found to be sensitive to Levofloxacin and Gatifloxacin. Total 90% were sensitive to Azithromycin followed by 80% to Doxycycline Hydrochloride and 50% to Cefixime (Table 3). Out of 10 isolates of *E. coli* when tested against 4 spices such as Black Pepper, Coriander, Garlic and Ginger, it was found that 50% *E. coli* were found to be sensitive to Garlic and Ginger each followed by 40% to Coriander and 30% to Black Pepper (Table 4).

Table 2. *E. coli* Contamination in Drinking Water Sample from Different Sources

Drinking Water Sample Source	No. of Water Samples Analyzed	*E. coli* Contaminated Water Samples
Tap Water	13	4 (30.76%)
Water Cooler	3	2 (66.66%)
Water Purifier	7	4 (57.14%)
UV Water Purifier	5	0
Total	28	10 (35.71%)

Table 3. Antibiotic Susceptibility test of *E. coli*

Antibiotics	No. of Resistant *E.coli*	
	Sensitive	Resistant
Azithromycin	9	1
Amoxyclav	0	10
Carbenicillin	0	10
Cefixime	5	5
Cefuroxime	0	10
Doxycycline Hydrochloride	8	2
Gatifloxacin	10	0
Levofloxacin	10	0

Table 4. Antimicrobial Activity of Spices Extract on *E. coli*

Drinking water (DW) Sample	Black Pepper	Coriander	Garlic	Ginger
DW1 (Tap Water)	R	11mm	14mm	16mm
DW2 (Water Purifier)	13mm	11mm	R	R
DW7 (Water Purifier)	R	R	R	R
DW9 (Water Purifier)	R	13mm	11mm	13mm
DW10 (Water Cooler)	R	R	R	R
DW11 (Tap Water)	R	R	13mm	13mm
DW14 (Tap Water)	13mm	12mm	13mm	12mm
DW23 (Tap Water)	12mm	R	R	11mm
DW24 (Water Cooler)	R	R	R	R
DW28 (Water Purifier)	R	R	14mm	R

Where, R= Resistant

DISCUSSION

The present study was conducted to evaluate the effect of different spices on drinking water *E. coli*. In the present study total 28 drinking water samples were analysed for the presence of *E. coli* out of which 10 samples were found to be contaminated with *E. coli*. The isolated *E.coli* were tested against 8 different antibiotics. It was found that all the isolates of *E. coli* (100%) were found to be resistant to Carbenicillin, Cefuroxime, and Amoxyclave while 100% *E. coli* found to be sensitive to Levofloxacin and Gatifloxacin. Total 90% were sensitive to Azithromycin followed by 80% to Doxycycline Hydrochloride and 50% to Cefixime. Out of 10 isolates of *E. coli* when tested against 4 spices such as Black Pepper, Coriander, Garlic and Ginger, it was found that 50% *E. coli* were found to be sensitive to Garlic and Ginger each followed by 40% to Coriander and 30% to Black Pepper. *A. sativum* has traditional dietary and medicinal applications as an anti-infective agent (Z.M. Ross *et al*., 2001). In vitro evidence of the antimicrobial activity of fresh and freeze- dried garlic extracts against many bacteria (L.P. Rees *et al*., 1993), fungi and viruses (N.D. Weber *et al*., 1992) supports these applications. Allicin, the active ingredient of *A. sativum*, acts by partially inhibiting DNA and protein synthesis and also totally inhibiting RNA synthesis as a primary target (M.E. Eja *et al*., 2007). Z. *officinale* has been used widely as herbal medicine. In particular, its gingerol-related components have been reported to possess antimicrobial and anti fungal properties, as well as several pharmaceutical properties (M. Park *et al*., 2008). *Coriandrum sativum* is considered both herb and spice since both its leaves and seeds are used as a seasoning condiment (N.M.A. Chaudhry and P. Tariq, 2006). It has traditionally been referred to as antimicrobial (I. Kubo *et al*., 2004). The seeds of *C. sativum* contain 0.5-1% essential oil and are rich in beneficial phytonutrients including carvone, geraniol, limonene, borneol, camphor, elemol and linalool (K. Isao *et al*., 2004). Ferrous sequestering activity of these compounds may play role in inhibiting microbial growth (P.Y.Y. Wong and D.D. Kitts, 2006). *P. nigrum* is used to treat asthma, chronic indigestion, colon toxins, obesity, sinus, congestion, intermittent fever, cold extremities, colic, gastric ailments and diarrhoea (Ao *et al*., 1998). It has shown to have antimicrobial activity (H.J.D. Dorman and S.G. Deans, 2000). It is interesting to note that even crude extracts of these spices showed antimicrobial activity against multidrug resistant *E. coli* where modern antibiotic therapy has limited effect. The effect of these spices on these organisms in vivo cannot be predicted from this study. Thus, there is a need for detailed scientific study of traditional medical practices to ensure that valuable therapeutic knowledge of some spices is preserved and also to provide scientific evidence for their efficacies.

Conclusion

In the present study it was concluded that the antibiotic resistant *E. coli* were sensitive to naturally available spices such as Black Pepper, Coriander, Garlic and Ginger. The extracts of these spices could be a possible source to obtain new and effective herbal medicines to treat diarrheal diseases caused by multi-drug resistant strains of *E. coli* in community. However, it is necessary to isolate the active constituents, and determine their toxicity, side effects and pharmacokinetic properties.

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