Antibacterial activity of natural spices on multiple drug resistant Escherichia coli isolated from drinking water, Bangladesh

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Abstract

Background: Spices traditionally have been used as coloring agents, flavoring agents, preservatives, food additives and medicine in Bangladesh. The present work aimed to find out the antimicrobial activity of natural spices on multi-drug resistant Escherichia coli isolates.

Methods: Anti-bacterial potentials of six crude plant extracts (Allium sativum, Zingiber officinale, Allium cepa, Coriandrum sativum, Piper nigrum and Citrus aurantifolia) were tested against five Escherichia coli isolated from potable water sources at kushtia, Bangladesh.

Results: All the bacterial isolates were susceptible to undiluted lime-juice. None of them were found to be susceptible against the aqueous extracts of garlic, onion, coriander, pepper and ginger alone. However, all the isolates were susceptible when subjected to 1:1:1 aqueous extract of lime, garlic and ginger. The highest inhibition zone was observed with lime (11 mm).

Conclusion: Natural spices might have anti-bacterial activity against enteric pathogens and could be used for prevention of diarrheal diseases. Further evaluation is necessary.

Introduction

Multi-drug resistant strains of Escherichia coli are widely distributed in hospitals and are increasingly being isolated from community [1]. Thus, it is urgent need to find out new antimicrobial agents. However, new families of antimicrobial agents will have a short life expectancy [2]. 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 [3].

Spice plants and essential oils extracted from them have become important due to their potential antimicrobial [4-6] and stimulating effects in the animal digestive system. 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 [7]. Spice plants have been used traditionally as coloring agents, flavoring agents, preservatives, food additives and as well as anti-parasitic, anthelmintic, analgesic, expectorant, sedative, antiseptic and anti-diabetic substances in many parts of the world [8]. In addition, they possess biological activities such as that of antioxidants [9] and hypocholesterolemic [10]. In this study, we aimed to explore the antibacterial effect of natural spices on multiple drug resistant E. coli isolates.

Materials and methods

Multi-drug resistant E. coli used in present study were isolated from drinking water of various region of kushtia town by standard methods [11]. Pure cultures of isolates were preserved at 4°C on nutrient agar slants. In order to confirm the identity of the test bacterial isolates morphological characteristics and conventional biochemical tests were performed according to Harley and Prescott [11]. The spices were bought from local market.
Garlic and onion were peeled, cut into pieces and sun-dried for one week. These were then grounded using a blender. Twenty gram of the ground material (garlic, onion, ginger, pepper and coriander seed) were soaked in 100 ml of hot sterile water and allowed to stand for 72 h. The lime fruits were washed with sterile water then cut open with a sterile knife and the juice pressed out. The crude extracts were obtained by filtration [12]. Sterile paper disks of 5 mm diameter were impregnated with 10 μl of crude extracts and mixtures of different crude extracts (1:1, 1:1:1), then dried in a hot air oven at 60°C for five min. Each disk contains approximately 2 μg of crude extract. All extracts were stored at 4°C when not in use.

Susceptibility testing
Standard norfloxacin (NOR, 10 μg/disc), clindamycin (CC, 2 μg/disc), neomycin (N, 30 μg/disc), cefoxitin (FOX, 30 μg/disc), streptomycin (S, 10 μg/disc), ciprofloxacin (CPX, 5 μg/disc), chloramphenicol (C, 30 mcg/disc), tetracycline (T, 30 mcg/disc), penicillin G (P, 10 units/disc), erythromycin (E, 15 mcg/disc), cefotaxime (CTX, 30 μg/disc), nalidixic acid (NA, 30 mcg/disc), cloxacillin (CX, 1 mcg/disc), kanamycin (K, 30 mcg/disc), amoxicillin (AM, 30 mcg/disc) were used for comparison of the antibacterial activity.

Results and discussion
Table 1 shows the antibiotic resistance pattern of the isolated strains. All strains showed resistance against the activity of clindamycin and cloxacillin. Penicillin G is also not quite effective against those isolates as four of the isolates are resistant against it. Highest growth inhibition zones were found with ciprofloxacin followed by cefotaxime and norfloxacin. The multiple antibiotic resistance of E. coli demonstrated in this study accord with those found in other studies [15-17].

Table 2 shows that the aqueous extracts of Allium sativum and Zingiber officinale alone and in combination (1:1) did not exhibit any in vitro inhibition on the growth of test organisms except in case of one isolate for later cases (7 mm). Similarly Allium cepa, Piper nigrum, and Coriandrum sativum seed alone did not exhibit any in vitro anti-bacterial effect, however the combination of Allium cepa + Allium sativum (1:1) and Citrus aurantifolia + Zingiber officinale + Allium sativum (1:1:1) showed inhibition zones. Citrus aurantifolia juice showed the highest effect on the test organisms. These findings are in good accord with others [12,18-22].

A. sativum has traditional dietary and medicinal applications as an anti-infective agent [23]. In vitro evidence of the antimicrobial activity of fresh and freeze-dried garlic extracts against many bacteria [24], fungi and viruses [25] 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 [26]. Like A. sativum, A. cepa is another medicinally important anti-infective agent [12]. Raw A. cepa can completely sterilize mouth and throat. Organosulfur compounds (OSC)s and phenolic compounds have been reported to be involved in the A. cepa antimicrobial activity [27]. 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 [28]. C. sativum is considered both herb and spice since both its leaves and seeds are used as a seasoning condiment [18]. It has traditionally been referred to as antimicrobial [29]. 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 [30]. Ferrous sequestering activity of these compounds may play role in inhibiting microbial growth [31]. P. nigrum is used to treat asthma, chronic indigestion, colon toxins, obesity, sinus, congestion, fever, intermittent fever,
cold extremities, colic, gastric ailments and diarrhoea [32]. It has shown to have antimicrobial activity [33]. From HPTLC analysis it has reported that the major phytochemical present in the crude extract of *P. nigrum* was found to be pipericine thus the inhibitor effect of crude extract of *P. nigrum* and its active constituent was found to be pipericine [34]. *C. aurantifolia* is popular as fruit and food ingredient for flavoring due to tannins, saponins, phenolic compounds, essential oils and flavonoids [37]. It is interesting to note that even crude extracts of these plants showed good activity against multidrug resistant strains where modern antibiotic therapy has limited effect.

The effect of these spices on these organisms in vivo cannot be predicted from this study. And though paper disk assays a practical approach to study potential antibacterial compounds, using the size of inhibition zone to indicate relative antibacterial activity is not adequate. The zone must be affected by the solubility and rate of diffusion in agar medium or its volatilization; and thus the results could be affected.

Thus, there is a need for detailed scientific study of traditional medical practices to ensure that valuable therapeutic knowledge of some plants is preserved and also to provide scientific evidence for their efficacies.

### Conclusions

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 pharmaco-kinetic properties.

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