Digest Journal of Nanomaterials and Biostructures Vol. 5, No 4, October-December 2010, p. 821-827

IN VITRO ANTIMICROBIAL EVALUATION OF COMMERCIAL TEA EXTRACTS AGAINST SOME PATHOGEN FUNGI AND BACTERIA

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The *in vitro* antimicrobial activity of crude ethanol, methanol, hexane and aqueous extracts of commercial tea types such as fennel (*Foeniculum sp.*), senna (*Cassia sp.*), basil-rosemary (*Ocimum sp.-Rosmarinus sp*), daisy (*Bellis sp.*) and sage (*Salvia sp*) against pathogen fungi (*Colletotrichum coccodes, Epicoccum nigrum, Scopulariopsis brevicaulis*) and foodborne pathogen bacteria (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus*, *Klebsiella pneumoniae*, *Enterobacter* sp.) were investigated with disk diffusion method. Senna (*Cassia sp.*) exhibed the most effective antimicrobial activity in both ethanolic and methanolic extracts. The best antimicrobial effect (30.6 mm) against *E. nigrium* was seen in methanolic senna (*Cassia sp.*) extract. The results of the study indicate that commercial tea types have showed various levels of antimicrobial activity depend on the type of solvent used in the extraction procedure.

(Received September 11, 2010; accepted October 13, 2010)

Keywords: Antibacterial activity, Antifungal activity, Tea extracts

1. Introduction

The main basis for the therapy of microbial (bacterial and fungal) infections is provided by antibiotics. There was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases since the discovery of these antibiotics and their uses as chemotherapeutic agents. Yet, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms [1]. The worldwide emergence of *Escherichia coli, Klebsiella pneumoniae* and many other ß-lactamase producers has become a major therapeutic problem. Multi-drug resistant strains of *E. coli* and *K. pneumoniae* are widely distributed in hospitals and are increasingly being isolated from community acquired infections [2]. All this has resulted in severe consequences including increased cost of medicines and mortality of patients [3].

Medicinal plants such as fennel, senna, basil, rosemary, sage and daisy which has potential antimicrobial properties is grown naturally in Turkey [4]. Some are produced by making the culture (fennel, daisy, basil, etc.). Due to being available naturally in the environment these plants are collected and consumed in different form by local people (brewing tea, spices, salad).

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Escherichia coli, Staphylococcus aureus, *Bacillus cereus*, *Klebsiella pneumoniae*, *Enterobacter* spp. are foodborne pathogens, found to be resistant to various antibiotics [5]. *Colletotrichum coccodes, Epicoccum nigrum* and *Scopulariopsis brevicaulis* are fungal pathogens also known as cause of asthma in humans. It has been proven by various studies that these fungal pathogens are resistant to various anti-fungal [6]. It is thought to be useful for public health that the development of new drugs against bacteria resistant to antibiotic and fungi resistant to anti-fungal resistance. According to the WHO, medicinal plants would be the best source for obtaining variety of drugs [3]. For the past two decades, there has been an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agent [7].

In this study, the in vitro antimicrobial activity of crude ethanolic, methanolic, hexane and aquatic extracts of the fennel (*Foeniculum sp.*), senna (*Cassia sp*.), basil-rosemary (*Ocimum sp.-Rosmarinus sp*), daisy (*Bellis sp.*) and sage (*Salvia sp*) against pathogen fungi (*Colletotrichum coccodes, Epicoccum nigrum, Scopulariopsis brevicaulis*) and bacteria (*Escherichia coli* ATCC 25922*, Staphylococcus aureus* ATCC 25923, *Bacillus cereus*, *Klebsiella pneumoniae*, *Enterobacter* spp.) were investigated by the disk diffusion method.

2. Experimental

2.1. Tea types and preparation of extracts

Some commercial tea types such as fennel (*Foeniculum* sp.), senna (*Cassia* sp.), basil-rosemary (*Ocimum* sp.-*Rosmarinus* sp), daisy (*Bellis* sp.) and sage (*Salvia* sp.) were used in this study. All tea types were purchased from local seller of medicinal herbs. Two grams (2.0 g) of the powdered tea material was soaked in 10 ml each of boiled tap water, 95% ethanol, methanol and hexane in separate 250 ml sterile conical flasks at room temperature with uniform shaking in a shaker for 12 h. The content was then filtered with a Whatman No. 1 filter paper. The filtrates were evaporated to dryness and then packed in separate clean dry bottles and stored at room temperature until required.

2.2. Test microorganisms

The test microorganisms used in this study (bacteria: *Escherichia coli* ATCC 25922*, Staphylococcus aureus* ATCC 25923, *Bacillus cereus*, *Klebsiella pneumoniae*, *Enterobacter* spp.; fungi: *Colletotrichum coccodes* ve *Epicoccum nigrum* ve *Scopulariopsis brevicaulis*) were obtained from the culture collections of the Research Hospital of Dicle University and Microbiology Research Laboratory, Department of Biology, Diyarbakir, Turkey. The bacterial isolates were first subcultured in a nutrient broth (NB) (Sigma) and incubated at 37°C for 24 h while the fungal isolates were subcultured on a Sabouraud Dextrose Agar (Sigma) for 72 h at 25°C.

2.3. Antibacterial activity

The disk diffusion method according to Collins et al. [8] was used to screen the antibacterial activity. Sterile blank disks (Oxoid) were impregnated with 50 µL of the tea extract. The bacterial cultures were inoculated on Nutrient Broth (Sigma) and incubated for 24 h at 37 ± 0.1°C. Adequate amounts of Nutrient Agar (Sigma) were dispensed into sterile plates and allowed to solidify under aseptic conditions. The counts of bacterial culture were adjusted to yield 10^7 - 10^8 cfu ml^{-1}, using the standard McFarland counting method. The test microorganisms (0.1 ml) were inoculated with a sterile spreader on the surface of solid medium in plates. The agar plates inoculated with the test microorganisms were incubated for 1 h before placing the extract

impregnated paper disk on the plates. The bacterial plates were incubated at 37 ± 0.1 °C for 24 h. After incubation, all plates were observed for zones of growth inhibition and the diameter of these zones was measured in millimeters. All tests were performed under sterile conditions in triplicate. Methanol, ethanol, hexane and boiled tap water were used as negative controls.

2.4. Antifungal activity

In vitro antifungal activity of the tea extracts was determined by the agar disk diffusion method according to Rubio et al. [9]. Briefly, a suspension of each tested fungus (10^5 cfu ml^{-1}) was carefully inoculated with a sterile spreader on the surface of solid medium in plates. The agar plates were incubated for 1 h before placing the extract impregnated paper disk on the plates. Sterile blank disk (Oxoid) were impregnated with 15 µl of each tested tea extract and placed on the inoculated plates. Disks with methanol, ethanol, hexane and boiled tap water were used as negative controls. These plates were incubated at 25ºC for 48 h. The diameters of the inhibition zones were measured in millimeters and their means were calculated. All the tests were performed in triplicate.

3. Results

Ethanolic extracts of the tea types tested showed varying degree of antimicrobial activities against the test microorganisms. Especially, ethanolic extracts of senna (*Cassia* sp.) showed antibacterial activities against *B. cereus*, *K. pneumoniae* and *Enterobacter spp*. The highest antibacterial activity (15.3 mm) was determined against *Enterobacter spp.* by ethanolic extract of senna (*Cassia* sp.) (Table 1).

Methanolic extracts of the senna (*Cassia* sp.) showed both antifungal and antibacterial activity. Methanolic extracts of the tea types tested showed especially antibacterial activity. Methanolic extract of senna (*Cassia* sp.) created the highest antimicrobial activity (30.6 mm) against *E. nigrium* (Table 2).

Hexane extract of tea types especially showed the antifungal activity. The highest antifungal activity (15.6 mm) was determined against *B. brevicaulis* by fennel. The highest antibacterial activity (10.6 mm) was determined against *K. pneumoniae* by hexanic extract of senna (*Cassia* sp.). Hexanic extracts of tea types has no effect on *C. coccodes* (Table 3).

It was determined that aquatic extracts of tea types especially showed antifungal activity. The highest antimicrobial activity by aquatic extract of sage (*Salvia* sp.) was 30.0 and 10.3 mm against *C. coccodes* and *B. cereus* respectively. Aquatic extract of tea types showed antibacterial effect only against *B. cereus* (Table 4).

Table 1. Antimicrobial effect of ethanol extracts on pathogen fungi and bacteria

	[a]DD							
	Pathogen Fungi			**Pathogen Bacteria**				
Tea types	*C. coccodes*	*E. nigrium*	*B. brevicaulis*	*E. coli*	*S. aureus*	*B. cereus*	*K. pneumoniae*	*Enterobacter spp.*
1	ND	ND	ND	ND	ND	ND	ND	ND
2	ND	ND	13.6±1.52	ND	ND	13.6±0.57	14.6±1.52	15.3±1.52
3	ND	ND	ND	ND	ND	ND	ND	11.3±1.15
4	ND	ND	ND	ND	ND	ND	9.33±1.15	10.0±1.00
5	ND	ND	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND	ND	ND

[a]DD, agar disk diffusion method. Diameter of inhibition zone (mm±SD).
1: Fennel (*Foeniculum sp.*), 2: Senna (*Cassia sp*.), 3: Basil-Rosemary (*Ocimum sp.-Rosmarinus sp*), 4: Daisy (*Bellis sp.),* 5: Sage (*Salvia sp.*), 6: blank disk impregnated with ethanol, n=3. ND = Not Determined (no zone of inhibition).

Table 2. Antimicrobial effect of methanol extracts on pathogen fungi and bacteria

	[a]DD							
	Pathogen Fungi			**Pathogen Bacteria**				
Tea types	*C. coccodes*	*E. nigrium*	*B. brevicaulis*	*E. coli*	*S. aureus*	*B. cereus*	*K. pneumoniae*	*Enterobacter spp.*
1	ND	ND	ND	ND	11.0±1.00	ND	ND	11.3±1.52
2	12.6±1.15	30.6±1.15	ND	ND	ND	11.6±0.57	13.3±1.52	14.3±0.57
3	ND	ND	ND	11.3±1.52	10.6±1.15	ND	8.6±1.15	10.3±2.51
4	ND	ND	ND	10.3±1.52	9.6±1.52	ND	9.3±1.52	10.3±1.52
5	ND	ND	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND	ND	ND

[a]DD, agar disk diffusion method. Diameter of inhibition zone (mm±SD).
1: Fennel (*Foeniculum sp.*), 2: Senna (*Cassia sp*.), 3: Basil-Rosemary (*Ocimum sp.-Rosmarinus sp*), 4: Daisy *(Bellis sp.),* 5: Sage (*Salvia sp.*), 6: blank disk impregnated with methanol**,** n=3. ND = Not Determined (no zone of inhibition).

Table 3. Antimicrobial effect of hexane extracts on pathogen fungi and bacteria

	[a]DD							
	Pathogen Fungi			**Pathogen Bacteria**				
Tea types	*C. coccodes*	*E. nigrium*	*B. brevicaulis*	*E. coli*	*S. aureus*	*B. cereus*	*K. pneumoniae*	*Enterobacter spp.*
1	ND	7.3±1.15	15.6±0.57	9.6±1.52	7.0±1.00	ND	ND	ND
2	ND	8.3±2.08	15.0±1.00	ND	ND	ND	10.6±1.15	9.3±1.15
3	ND	ND	7.0±1.00	ND	ND	ND	ND	ND
4	ND	8.6±1.52	ND	ND	5.6±1.52	ND	ND	ND
5	ND	10.6±1.52	11.0±1.00	ND	6.6±1.15	7.0±1.00	ND	ND
6	ND	ND	ND	ND	ND	ND	ND	ND

[a]DD, agar disk diffusion method. Diameter of inhibition zone (mm±SD).
1: Fennel (*Foeniculum sp.*), 2: Senna (*Cassia sp.*), 3: Basil-Rosemary (*Ocimum sp.-Rosmarinus sp*), 4: Daisy (*Bellis sp.),* 5: Sage (*Salvia sp.*), 6: blank disk impregnated with hexane, n=3. ND = Not Determined (no zone of inhibition).

Table 4. Antimicrobial effect of aqueous extracts on pathogen fungi and bacteria

	[a]DD							
	Pathogen Fungi			**Pathogen Bacteria**				
Tea types	*C. coccodes*	*E. nigrium*	*B. brevicaulis*	*E. coli*	*S. aureus*	*B. cereus*	*K. pneumoniae*	*Enterobacter spp.*
1	15.6±1.15	ND	ND	ND	ND	ND	ND	ND
2	ND	7.0±1.73	ND	ND	ND	ND	ND	ND
3	9.3±0.57	5.6±0.57	9.3±0.57	ND	ND	6.6±0.57	ND	ND
4	ND	6.6±0.57	ND	ND	ND	ND	ND	ND
5	30.0±1.00	6.6±1.15	14.6±1.52	ND	ND	10.3±1.52	ND	ND
6	ND	ND	ND	ND	ND	ND	ND	ND

[a]DD, agar disk diffusion method. Diameter of inhibition zone (mm±SD).
1: Fennel (*Foeniculum sp.*), 2: Senna (*Cassia sp.*), 3: Basil-Rosemary (*Ocimum sp.-Rosmarinus sp*), 4: Daisy (*Bellis sp.),* 5: Sage (*Salvia sp.*), 6: blank disk impregnated with boiled tap water, n=3. ND = Not Determined (no zone of inhibition).

4. Discussion

The inhibitory effects of aqueous and methanolic extracts of medicinal plants have been reported [10,11]. All the extracts from the different solvents demonstrated antimicrobial activity. Variation in activity among different extracting solvents has earlier been reported [12]. The little or no antibacterial activities of the aqueous extract against most bacterial strains investigated in this study is in agreement with previous works which show that aqueous extracts of plant generally showed little antibacterial activities [13,14].

In this study, hexane, ethanol, methanol and aqueous extracts of *Cassia sp*. (senna) leaves were prepared and antimicrobial activity were measured by the method of disk diffusion. It was determined that hexane, ethanol, methanol extracts formed a significant inhibition zone for Gram-negative bacteria, and that aqueous extracts had no antibacterial effect. Duraipandiyan and Ignacimuthu, [15] identified inhibition zones of hexane, chloroform, ethyl acetate, methanol and water extracts of *Cassia fistula* flowers against Gram positive and Gram-negative bacteria in their study. According to the results of their study, extracts have antimicrobial activity against Gram-negative and Gram-positive bacteria, and these support our work.

Hexane, methanol, ethanol and aqueous extracts of *Rosmarinus officinalis-Ocimum basilicum* we were prepared and it was determined that methanol extracts of *Rosmarinus officinalis- Ocimum basilicum* formed a significant inhibition zone for Gram-negative bacteria *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Enterobacter* spp. Celiktas et al. [16] determined that antimicrobial activity of methanol extract of *Rosmarinus officinalis* by disk diffusion method in their study. They found that in microorganisms they used, *S. aureus* was more sensitive than other microorganisms.

In our study, it was determined that the extract of *Salvia* sp. that was prepared with different solutions did not have antibacterial effects for *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* spp. Kelen et al. [17] investigated antimicrobial and antioxidant effects of essential oils of *Salvia* species in Turkey, and they found *B. cereus, K. pneumoniae* and *E. coli* showed resistance to salvia oils, which supports our work.

5. Conclusions

The organisms used for the purpose of this investigation are associated with various forms of infections; *E. coli* (gastrointestinal tract infections), *S. aureus* (skin infections and septicaemia), *B. cereus* (wound infections), *Enterobacter* (respiratory tract infections, skin and soft-tissue infections etc.) [18], *K. pneumoniae* (pneumonia) [19], *C. coccodes*, *E. nigrum*, *S. brevicaulis* (many plants and human diseases) [20-22]. Results of this investigation therefore have shown that all the tea extracts from the different solvents are a potential source of antibiotic or antifungal substances for drug development for use against this group of pathogens.

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