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Preliminary communication

ANTIBACTERIAL EFFECT OF SPROUTS AGAINST HUMAN PATHOGENS IN VITRO

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Sprouts contain minerals, vitamins, and other compounds, which may have antimicrobial activity. Antimicrobial compounds are released from homogenized sprouts and diffuse into the culture medium inhibiting the growth of pathogenic bacteria. These antibacterial compounds may influence the multiplication of contaminating pathogens. In this study the antimicrobial effects of 55 different homogenized sprouts were investigated with agar well diffusion method on human pathogenic bacteria. Homogenizates of different radish, early kohlrabi, and red cabbage sprouts caused inhibitory zone around the wells on the surface of inoculated agar plates. Mustard, zucchini, medical (German) chamomile, spicy fenugreek, and adzuki bean sprouts had antimicrobial effect only against a few human pathogenic bacteria. Twenty-nine other spicy and vegetable sprouts had no antibacterial activity against the investigated human pathogens. The results suggest that a few sprouts have antimicrobial properties, but different cultivars of the same species have different effects against different bacterial strains. The sprouts of radish cultivars contained the most effective antibacterial compounds.

Keywords: sprouts, antimicrobial effect, pathogenic bacteria

Sprouts are used in modern cooking as they can be added to salads, sandwiches, and other dishes. Sprouting is one of the easiest ways to obtain fresh vegetables for eating in each season. Fresh sprouts can be ideal sources of vitamins, dietary fibres, and minerals for humans and for microorganisms (KUMAR & CHAUHAN, 1993; ZIELINSKI et al., 2005).

Previous studies have already described microbial contamination of sprouts (ROBERTSON et al., 2002). There are sprouts, which can serve as a good culture media for bacteria, so some papers blamed sprouts that may have been contaminated by enteric pathogens for food-borne infections. There were described outbreaks caused by enteric pathogens, like *Salmonella,* enterohaemorrhagic *Escherichia coli* (EHEC), and Shiga toxin-producing *E. coli* (STEC), contaminated sprouts in several countries (TAORMINA et al., 1999; FRANK et al., 2011). Alfalfa sprouts were most frequently contaminated, followed by beans.

However, some studies described that some plants, seeds, and sprouts have antimicrobial activity against plant pathogenic bacteria and fungi. Small cysteine-rich antifungal protein

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Raphanus sativus-antifungal protein 1 (Rs-AFP1 also called plant defensin) can be released from sprouted radish seed (Terras et al., 1995). Other plants also produce defensins, but only a few hane antimicrobial activity as radish and sweet potato (Huang et al., 2008). These small proteins (45–54 amino acids) inhibit the growth of fungi (Terras et al., 1995; Thomma et al., 2002). The concentration of plant defensins is higher in the seeds and sprouted seeds than in mature plant and may play a role in the protection of seeds (Osborn et al., 1995). Antimicrobial activity of sulphur compounds (methyl methane-thiosulfinate, sulforaphane, and allicin) were determined in the mature plant of the members of the *Brassicacaea* and *Allium* families (Kyung & Lee, 2001). Components of many seeds and plants other than sprouts are known to have antimicrobial activity against human pathogens. Sulforaphane compounds extracted from broccoli have an excellent antimicrobial effect on human pathogen *Helicobacter pylori* (Yanaka et al., 2009; Moon et al., 2010). Chloroform extract of wheat germ has shown antimicrobial activity against *Staphylococcus aureus* and *Bacillus cereus* (Kim et al., 2010). The antibacterial activity of sprouts has rarely been reported. In our study, the effects of homogenized sprouts of different plants were investigated on human pathogenic bacteria in vitro.

1. Materials and methods

1.1. Seed samples

Fifty-five (55) different vegetable and spicy seeds (Table 1) were purchased from the Vegetable Crops Research Institute Co. Ltd (Kecskemét, Hungary), Redei Kertimag Seedtrading Plc. (Rede, Hungary), and Szentesi-Mag Ltd (Szentes, Hungary). Seeds were cleaned with sterile distilled water before germination.

Table 1. Sprouts of plants used in this study

UMBELLIFERRAE (APIACEAE)	SOLANACEAE	CRUCIFERAE (BRASSICACEAE)	CUCURBITACEAE	FABACEAE
Carrot (*Daucus carota* L. subsp. *sativus)*	**Tomato** (*Licopersicon esculentum* L.)	**Brussel sprout** (*Brassica oleracea* L. convar. *oleracea* var. *gemmifera* DC.)	**Chives** (*Allium schoenoprasum* L.)	**Bean** (*Phaseolus vulgaris* L.)
Parsley (*Petroselium crispum* (Mill.) Fuss convar. *tuberosum)*	**Eggplant** (*Solanum melogena* L.)	**Savoy cabbage** (*Brassica oleracea* L. convar. *capitata* var. *sabauda*)	**Shallot** (*Allium ascolicum*) Hort.	**Azuki beans** (*Phaseolus/Vigna angularis* (Willd.) Ohwi et Ohashi var. *angularis)*
Parsley (*Petroselium crispum* (Mill.) Fuss convar. *crispum)*	**Pepper** (*Capsicum annuum* L. var. *longum*)	**Red cabbage** (*Brassica oleracea* L. convar. *capitata* var. *rubra* DC)	**CUCURBITACEAE**	**Chickpea** (*Cicer aretinum* L.)
Celery (*Apium graveolens* L.convar. *rapaceum)*	**"Almapaprika"** *(Capsicum annuum* L. var. *grossum)*	**Kohlrabi** (*Brassica oleracea* L. convar. *acephala (*DC.*)* var. *gongyl)*	**Cucumber** (*Cucumus sativus* L.)	**Lentil** (*Lens culinaris* Medik)

UMBELLIFERAE (APIACEAE)	**SOLANACEAE**	**CRUCIFERAE (BRASSICACEAE)**	**CUCURBITACEAE**	**FABACEAE**
Chervil (*Anthricus cerefolium* L. Hoffm.)	**Cherry pepper** (*Capsicum annuum* L. var. *cerasiforme*	**Broccoli** (*Brassica oleracea* L. convar. *botrytis* L. var. *cymosa* Duch)	**Zucchini** (*Cucurbita pepo* L.)	**Sicklefruit fenugreek** (*Trigonella foenum-graecum* L.)
Anise (*Pimpinella anisum* L.)	**Pritamin pepper** (*Capsicum annuum* L. var. *lycopersiciforme*)	**Cauliflower** (*Brassica oleracea* L. convar. *botrytis* L. provar. *botrytis* L.)	**Patisson** (*Cucurbita pepo* L. var. *patissonina*)	**LABIATAE (LAMIACEAE)**
Caraway (*Carum carvi* L.)	**COMPOSITAE (ASTERACEAE) (Tubuliflorae)**	**Garden cress** (*Lepidium sativum* L.)	**Asian pumpkin /fig-leaf gourd, Bouché/** (*Cucurbita ficifolia*)	**Origano** (*Origanum vulgare* L.)
Fennel (*Foeniculum vulgare* Miller)	**Sunflower** (*Helianthus annuus* L.)	**Rocket** (*Eruca vesicaria* L. Cav subsp. *sativa*)	**Melon** (*Cucumis melo* L.)	**Hyssop** (*Hyssopus officinalis* L.)
CHENOPODIACEAE	**German chamomile** (*Matricaria recutita* L.)	**Mustard** (*Sinapsis alba*)	**Watermelon** (*Citrullus lanatus* Thunb.)	**Marjoram** (*Origanum majorana* L.)
Beetroot (*Beta vulgaris* L. subsp. *esculenta* convar. *crassa* provar. *conditiva* Alef.)	**Wormwood** (*Artemisia absinthium* L.)	**Spring radish** (*Raphanus sativus* L. var. *sativus* convar. *radicula* subsp. *sativus 'Riesenbutter*)	**GRAMINEAE**	**IRIDACEAE**
Foliage beet or spinach beet *(Beta vulgaris* convar. (subsp.) *vulgaris)*	**Black salsify or scorzonera** (*Scorzonera hispanica* L.)	**Black radish** **(***Raphanus sativus* L. subsp. *niger* var. *niger)*	**Corn** (*Zea mays* L.)	**Saffron** *(Crocus sativus* L.*)*
	Iceberg lettuce (*Lactuca sativa* L.)	**Winter icicle radish (Daikon)** (*Raphanus sativus* L. var. *longipinnatus*)	**Wheat** (*Triticium aestivum* L.)	
	Escarole (*Cichorium endivia* L.)	**German Munich beer radish** **(***Raphanus sativus* L. subsp. *niger* var. *albus* 'Munchen bier'*)*		
		Summer radish *(Raphanus sativus* L. convar. *sativus* 'Osterguss rosa'*)*		

1.2. Seed germination

The seeds were layered on autoclaved filter paper covered cotton, which was soaked in tap water and placed in a plastic box. Seeds were sprouted for 1 week at room temperature.

Covering the box with aluminium folia provided moisturized atmosphere. Sprouts were harvested (were cut above the root) by sterile scissors and forceps, collected in sterile 50 ml plastic tubes, and homogenized by T25 basic dispenser (IKA® Labortechnik).

1.3. Inoculum preparation

Five (5) human enteric pathogenic bacteria – enterotoxic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), *Salmonella Typhimurium, Shigella flexneri* – clinical isolates, four members of *Enterobacteriaceae E. coli* ATCC 25922, *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Enterobacter* sp.– clinical isolates, *Pseudomonas aeruginosa* ATCC 27853, *S. aureus* ATCC 23923, and methicillin resistant *S. aureus* (MRSA) – clinical isolate strains were applied. The bacterial strains were cultured in Mueller-Hinton broth (BIOLAB Inc, Hungary) at 37 °C until McFarland 0.5 turbidity standard was reached. The individual bacterial suspensions were diluted to approx. 10^6 colony forming unit (CFU ml^{-1}) for the agar diffusion assay.

1.4. Screening the antimicrobial effect of homogenized sprouts

Antimicrobial effects of homogenized sprouts were investigated with agar well diffusion method. Sterile cotton swabs were wetted with appropriately diluted bacterial suspension of the different strains and were spread on punched Mueller–Hinton agar (Bio-Rad Hungary Ltd) plates individually. Then the wells were filled with different homogenized sprouts using sterile loops and forceps. Physiological saline (sodium-chloride solution of 0.9%) was applied as negative control, and 10 mg imipenem (for Gram-negative bacteria) and 30 mg vancomycin (against MRSA) in 100–100 ml distilled water were used as positive control. The well's size was approximately 150 mm^3. The plates were incubated at 37 °C for 24 h. The diameters of inhibitory zones were determined. The inhibitory zones developed around the filled wells mean antimicrobial effects of the given sprouts. Three samples of each sprout were investigated.

1.5. Survival of pathogens in tenfold dilution of radish sprout

One (1) g of homogenized radish sprout was diluted to 1:9 in tryptic soya broth (TSB) (BIOLAB Inc, Hungary) and was inoculated with 10^2 CFU g^{-1} of EHEC strain. TSB contaminated with the same amount of bacteria and 1 g of sprouts diluted in 9 ml TSB were applied as positive and negative controls, respectively. Hundred μl of each suspension was spread on sorbitol MacConkey agar plate after the inoculation and after 24 h incubation at 37 °C. Colonies on sorbitol MacConkey agar (BIOLAB Inc, Hungary) were checked after 24 h incubation at 37 °C.

2. Results and discussion

Homogenized sprouted radish, kohlrabi, and red cabbage cultivars showed inhibitory zones around the wells on the inoculated agar surface for most of the examined bacterial strains. Sprouts of different cultivars of radish had different impact on the growth of pathogenic bacteria (Table 2). Mustard, zucchini, chamomile, spicy fenugreek, and adzuki bean sprouts only had antibacterial effect against *S. aureus* strains and a few Gram-negative species, while they had no impact on the other investigated human pathogenic bacteria. Twenty-nine other spice and vegetable sprouts (Table 3) had no antibacterial activity against the investigated human pathogens. No inhibitory zone could be detected for any sprouts on the plates inoculated by *P. aeruginosa.*

Table 2. Antimicrobial activities of sprouts of different vegetables and spices

	Kohl-rabi	Red cabbage	Mustard	Radish Oster-gruss rosa	Black radish	Winter radish (icicle)	Munich beer radish	Spicy fenu-greek	Black salsify	Zuc-chini
ETEC	++	+	–	+	++	+	++	–	–	–
EIEC	++	+	–	+	++	+	++	+	–	–
EHEC	+	+	+	+	++	–	++	–	–	–
S. Typhimu-rium	++	+	+	+	++	+	++	–	+	–
S. flexneri	++	+	–	+	++	+	++	–	–	–
E. coli ATCC 25922	++	+	–	+	++	–	++	++	–	–
P. mirabilis	++	+	–	+	++	+	++	–	–	–
K. pneumo-niae	+	+	–	+	+	+	++	–	–	–
Enterobacter sp.	++	+	–	++	++	+	++	+	+	–
S. aureus ATCC 25923	++	+	+	+	++	–	++	++	–	+
MRSA	++	+	+	+	++	–	++	+	–	+
P. aerugi-nosa ATCC 27387	–	–	–	–	–	–	–	–	–	–

+ : Diameter of inhibitory zone 9–12 mm; ++: diameter of inhibitory zone 12–22 mm; –: no detected inhibitory zone

Table 3. Vegetable and spicy sprouts that have no antimicrobial effect against the investigated bacteria

Carrot (*Daucus carota* subsp. *sativus)*	Beetroot (*Beta vulgaris* L. subsp. *esculenta* convar. *crassa* provar. *conditiva)*	Sunflower (*Helianthus annuus)*	Corn (*Zea mays*)
Parsley (*Petroselinum crispum* convar. *tuberosum)*	Foliage beet or spinach beet *(Beta vulgaris* convar. (subsp.) *vulgaris)*	Escarole (*Cichorium endivia)*	Wheat (*Triticium aestivum*)
Parsley (*Petroselinum crispum* convar. *crispum)*	Tomato (*Lycopersicon esculentum)*	Chives (*Allium schoeno-prasum)*	Origano (*Origanum vulgare*)
Celery (*Apium graveolens* convar. *rapaceum)*	Eggplant (*Solanum melongena)*	Shallot (*Allium ascaloni-cum)*	Marjoram (*Origanum majorana*)
Chervil (*Anthricus cerefolium*)	Pepper (*Capsicum annuum* var. *longum*)	Cucumber (*Cucumus sativus)*	Saffron (Crocus sativus)
Anise (*Pimpinella anisum*)	"Almapaprika" *(Capsicum annuu*m var. *grossum)*	Asian pumpkin/fig-leaf gourd (*Cucurbita ficifolia* Bouché)	
Caraway (*Carum carvi)*	Cherry pepper (*Capsicum annuum* var. *cerasiforme)*	Melon (*Cucumis melo*)	
Fennel (*Foeniculum vulgare*)	Pritamin pepper *(Capsicum annuum* var. *lycopersiciforme)*	Watermelon (*Citrullus lanatus* Thunb.)	

Our results suggest that radish (*Raphanus sativus* L. 'Osterguss rosa 2'), black radish (*Raphanus sativus* subsp. *niger* var. *niger*), Munchen beer radish (*Raphanus sativus* L. subsp. *niger* var. *albus* 'Munchen bier'), kohlrabi (*Brassica oleracea* L. convar *acephala* DC. var. *gongyl*), and red cabbage (*Brassica oleracea* L. convar. *capitata* var. *rubra* DC.) sprouts have antimicrobial activity against most of the investigated human pathogenic bacteria except *P. aeruginosa*. The size of inhibitory zones depended on the sprouted plant species (also cultivars) and the bacterial strains.

On the other hand, as any food component, sprouts may be contaminated with bacteria as well. It has already been described that different enteric pathogenic bacteria as *Salmonella* sp.*,* EHEC, and STEC strains contaminated the sprouts (mainly alfalfa) and caused food-borne infections and outbreaks among the consumers (Taormina et al., 1999; Buchholz et al.*,* 2011). We found that sprouts of different radish cultivars have good antibacterial effect against the investigated bacteria, but winter radish (also called icicle and daikon) (*Raphanus sativus* var. *longipinnatus*) has not inhibited the growth of EHEC, MRSA, and *S. aureus* strains. In Japan radish sprout was blamed to be the transmitter of EHEC outbreaks twice because the sprouts of daikon are very popular in Japanese cuisine and they add daikon radish sprouts (kaiware daikon) to sushi, soups, or salads. In two outbreaks EHEC strains were only isolated from the faeces of patients, but not from the consumed radish sprouts (National Institute of Infectious Diseases and Infectious Diseases Control Division, Ministry of Health and Welfare of Japan, 1997). Later Japanese researchers have demonstrated the presence of viable enterohaemorrhagic *E. coli* O157:H7 not only on the outer surfaces but also in the inner tissues of radish sprouts grown from seeds that were contaminated (Itoh et al., 1998). When the radish sprouts were of daikon radish, it could not inhibit the EHEC strains. It was retrospectively concluded from the analyses of consumed food data that probably the sprouts had been contaminated. STEC strain O104:H4 caused outbreaks in Germany in 2011 (Buchholz et al., 2011). In this infection the estimated median incubation period was 8 days (Frank et al. & HUS Investigation Team, 2011), which made it difficult to find the source of the infection, but again radish sprout was blamed first, fenugreek sprouts later. The outbreak in Germany was in early summer, so early type radish sprouts (*Raphanus sativus* L. var. *sativus* convar. *radicula)* may had been used, which has an inhibitory effect on pathogenic *E. coli.*

When the pathogenic bacterial contamination level is very low, probably sprouts with antibacterial properties in chewed form may inhibit the growth of pathogens. Naturally, other food or drink consumed together with the sprouts can dilute the antimicrobial compounds, which may decrease their activity. This was confirmed by our results, when EHEC strains survived in homogenized radish sprouts diluted tenfold.

Many researchers have published the methods how sprouts could be decontaminated and secure to eat (Bari et al., 2008, 2009; Waje et al., 2009; Nei et al., 2011).

We demonstrated that some sprouts have antimicrobial activity against human pathogens. Probably those compounds detected in the mature plant are already present in the sprouted form and show similar effect on bacteria.

Our study also showed that there are many sprouts, which have no antimicrobial activity against enteric pathogens. Outbreaks of food-borne infection caused by contaminated sprouted seeds of different plants would happen in the future, first we recommend investigating those sprouts, which have no antimicrobial effect against enteric bacterial pathogens. Our results suggest that sprouts of the *Brassicacaea* family members as kohlrabi, red cabbage, and radish (few varieties) sprouted at home can be eaten safe after rinsing with clean water if the hygienic rules are kept. Disinfection treatments are recommended for commercial and imported sprouts. A disinfectant method should be used that kills bacteria inside the plant tissue.

3. Conclusion

Despite the fact that some sprouts have good antibacterial property bacteria might be isolated from food containing those sprouts. Though antibacterial compounds are produced when sprouts are cut or chewed, bacteria contaminating the sprouts may be the vehicles of food-borne infection because of the diluting effect of other consumed food ingredients. However, 52% of the examined homogenized sprouts did not have antimicrobial effect. Hygienic measures must be kept even when working with sprouts of antibacterial properties. Effective disinfection, with electron beam for example, is recommended for the treatment of imported and commercial sprouts before marketing (Waje et al., 2009).

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