

# ANTIMICROBIAL ACTIVITY OF BUCKWHEAT (*Fagopyrum esculentum Moench*) HULLS EXTRACT



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**Abstract:** Many naturally occurring compounds found in plants, herbs, and spices have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against food borne pathogens. Their systematic screening may result in the discovery of novel effective antimicrobial compounds. The aim of this work was to test antibacterial activity of buckwheat hulls extract against the three species of Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative bacteria (*Salmonella choleraesuis*, *Escherichia coli* and *Proteus mirabilis*). Antimicrobial activity of buckwheat hulls extract was tested by the disc diffusion method according to the National Committee for Clinical Laboratory Standards Guidelines. The results indicated that the tested bacteria were sensitive in higher concentrations of buckwheat hulls extract.

**Key words:** Antimicrobial activity, buckwheat hulls extract

## INTRODUCTION

In spite of modern improvements in food production techniques, food safety is an increasingly important public health issue (WHO, 2002). Illnesses caused due to the consumption of foods contaminated with pathogens have a wide economic and public health impact worldwide (Gandhi & Chikindas, 2007). Therefore, there is need for new methods of reducing or eliminating food borne pathogens, possibly in combination with existing methods. One such possibility is the use of plant extracts or essential oils as antibacterial additives.

Many naturally occurring compounds found in plants, herbs, and spices have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against food borne pathogens (Kotzekidou et al., 2007). Their systematic screening may result in the discovery of novel effective antimicrobial compounds.

Consumer demand for reduced usage of synthetic preservatives has led to research and use of "naturally derived" antimicrobials. In modern food industries mild pro-

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cesses are applied in order to obtain safe products which have a natural or “green” image. Under these conditions the antimicrobial effects of plant extracts and essential oils intend to reduce the proliferation of food borne pathogens (Burt, 2004).

Common buckwheat (*Fagopyrum esculentum* Moench) is recognized as a healthy food in many countries because it is rich in flavonoids, vitamins, amino acids and other substances (Wijngaard & Arendt, 2006). Buckwheat seeds, classified as a pseudo-cereal, are recognized as a suitable component of food products due to their high nutritional value as well as antioxidant activity (Li and Zhang, 2001). The hulls removed before utilization of the seeds represent a new source of hemicelluloses, which nowadays are considered potential biopolymers for food and nonfood applications (Ebringerová and Heinze, 2000; Ebringerová and Hromádková, 1999). Buckwheat hulls are also rich in antioxidants comprising tocopherols, rutin, quercetin derivatives, and other phenolic substances (Watanabe, 1998; Hromádková et al., 2005). The hulls are more abundant in total phenolics and flavonoids in comparison to other parts of buckwheat grain (Quettier-Deleu et al., 2000). The antimicrobial activity of phenolic substances has been widely reported (Hattori et al., 1998; Cushnie & Lamb, 2005).

The aim of this study was to investigate antimicrobial effects of buckwheat hulls extract on three species of Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative bacteria (*Salmonella choleraesuis*, *Escherichia coli* and *Proteus mirabilis*).

## 1. MATERIALS AND METHODS

### 1.1. Plant material

Buckwheat (*Fagopyrum esculentum* Moench) hulls were purchased from Hemija commerce, Novi Sad, Serbia.

### 1.2. Extraction procedure

Buckwheat hulls sample (50 g) was mixed with 400 mL ethanol/water (80/20, v/v). Extraction was carried out at room temperature for 24 h, ultrasonicated for 10 min and supernatant was filtered through the

filter paper (Whatman, Grade 4 Chr, UK). The procedure was repeated twice. Combined extracts were dried by vacuum-evaporator. The dried extract was resolved in ethanol/water (80/20, v/v) to obtain concentration 100 mg/mL. The extract obtained by this procedure was used for further investigations of antimicrobial activity.

### 1.3. Microbial strains

Test microorganism which were used in this experiment are *Bacillus cereus* ATCC /10876/, *Staphylococcus aureus* ATCC /11632/, *Enterococcus faecalis* ATCC /14506/, *Escherichia coli* ATCC /10536 /, *Salmonella choleraesuis* ATCC /10708/ and *Proteus mirabilis* ATCC /12453/.

### 1.4. Determination of inhibitory activity by paper disc diffusion method

Antimicrobial activity of buckwheat hulls extract was tested by the disc diffusion method according to the National Committee for Clinical Laboratory Standards Guidelines (National Committee for Clinical Laboratory Standard, 1999). Buckwheat hulls extract solution was prepared in 80% ethanol geometric dilutions, ranging from 6.25 to 100 mg/mL of the extract. Sterilized filter paper discs Whatman (7 mm diameter) were soaked with 10 µl solution ranging from 6.25 to 100 mg/mL of buckwheat hulls extract. Plates were left for 30 min at room temperature to allow the diffusion of extract and then they were incubated at 37 °C for 24 h. The diameters of the inhibition zones were measured in millimeters. Studies were performed in triplicate and the developed inhibition zones were compared with scale. The measurement scale was the following (disc diameter included): ≥18 mm zone of inhibition is strongly inhibitory activity (+++); 13-18 mm zone of inhibition is moderately inhibitory activity (++); 10-13 mm zone of inhibition is intermediate inhibitory activity (+); and <10 mm is no inhibitory activity (Ratajac, 2006). Negative controls were prepared using the same solvent volumes to dissolve plant extract. All analysis were performed in triplicate, and the mean values with the standard deviations (SD) are reported.

## 2. RESULTS AND DISCUSSION

The plant extracts show strong activity if inhibition zone is  $\geq 18$  mm, moderate activity if inhibition zone is 13-18 mm, intermediate if inhibition zone is 10-13 mm and no inhibition if inhibition zone is  $<10$  mm. Buckwheat hulls extract was tested in four concentrations, ranging from 6.25 to 100 mg/mL of the extract. The results shown in Table 1 indicate that analyzed extract had different antimicrobial effect depending on applied bacterial strain.

Tested buckwheat hulls extract showed antimicrobial activity on all selected strains by screening with disc diffusion method. Buckwheat hulls extract exhibited higher antimicrobial activity against Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis*) than Gram-negative bacteria (*Salmonella choleraesuis*, *Escherichia coli*, *Proteus mirabilis*). Antimicrobial activity could be attributed to the presence of flavonoids in buckwheat hulls (Rodríguez et al., 2010). Since flavonoids are known to be synthesized by plants in response to microbial infection (Dixon, et al., 1983), it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. The position and number of hydroxyl groups of polyphenols mainly influence their inhibitory effect on microorganisms. There is an evidence that highly oxidized phenols possess more inhibitory action (Ramar & Ponnampalam, 2008). The mechanism responsible for phenolic activity to microorganisms also includes enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more non-specific interactions with proteins (Mason & Wasserman, 1987). Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Ramar & Ponnampalam, 2008). There is also a specific lipophilic flavonoid that may disrupt microbial membranes (Tsuchiya et al., 1996).

Gram-positive bacteria were determined to be more susceptible to the antimicrobial properties of buckwheat hulls extract than Gram-negative bacteria and it is considered to be due to its outer membrane. The activity varies with concentration of buckwheat hulls extract and bacteria species. These differences in the susceptibility of the test organisms to buckwheat hulls extract could be attributed to the rate of penetration of its constituent's structures through the cell wall and cell membrane. The ability of buckwheat extract to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control are the most likely reasons for its lethal action (Cox et al., 2001).

Concentration of 100 mg/mL of buckwheat hulls extract exhibited moderate antimicrobial activity against Gram-positive bacteria (Table 1). Concentration of 100 mg/mL of buckwheat hulls extract was the most efficient against *Bacillus cereus* (inhibition zone was  $13.9 \pm 0.33$  mm). For the same concentration of buckwheat hulls extract, *Enterococcus faecalis* exhibited lower inhibition zone of  $13.6 \pm 0.57$  mm, while *Staphylococcus aureus* exhibited the lowest sensitivity at this concentration ( $12.6 \pm 0.66$  mm). For the same concentration of buckwheat hulls extract (100 mg/mL) Gram-negative bacteria achieved lower inhibition zones.

Buckwheat hulls extract in concentration of 50 mg/mL produced the same inhibition zone against *Bacillus cereus* and *Enterococcus faecalis*,  $13.3 \pm 0.88$  mm and  $13.3 \pm 0.57$  mm, respectively. Lower inhibition zone ( $11.6 \pm 0.88$  mm) for *Staphylococcus aureus* was achieved. The same concentration of buckwheat hulls extract (50 mg/mL) resulted in lower inhibition zones for Gram-negative bacteria.

The extract concentrations lower than 50 mg/mL did not influence any of the tested microorganisms. Having insight in all our results, there is possibility to use buckwheat hulls extract against Gram-positive bacteria, but in higher concentration.

**Table 1.**  
Antimicrobial activity of buckwheat hulls extract

Concentration (mg/mL)	Inhibition zones (mm)					
	Gram - positive bacteria			Gram - negative bacteria		
	<i>S. aureus</i>	<i>B. cereus</i>	<i>E. faecalis</i>	<i>S. choleraesuis</i>	<i>P. mirabilis</i>	<i>E. coli</i>
100	12.6 ± 0.66	13.9 ± 0.33	13.6 ± 0.57	11.33 ± 0.33	11.0 ± 0.33	10.6 ± 0.33
50	11.6 ± 0.88	13.3 ± 0.88	13.3 ± 0.57	10.0 ± 0.57	10.33 ± 0.57	9.6 ± 0.33
25	n.i	n.i	n.i	n.i	n.i	n.i
12.5	n.i	n.i	n.i	n.i	n.i	n.i
6.25	n.i	n.i	n.i	n.i	n.i	n.i
k	n.i	n.i	n.i	n.i	n.i	n.i

n.i – no inhibition

### 3. CONCLUSION

Presented results suggest that buckwheat hull is potential source of compounds with antimicrobial activity against bacterial strains in food. Our results indicate that buckwheat hulls extract may be an ideal candidate for possible food preservation by natural plant-based products. Further research is necessary to determine the identity of the antibacterial compounds from buckwheat hulls and also to determine their full spectrum of efficacy. Furthermore, different extraction solvents and procedures could be investigated.

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