

## Antimicrobial Activities of the Edible Bird's Nest Extracts Against Food-borne Pathogens

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### Abstract

Antimicrobial activities of extracts from the edible bird's nest (EBN) against four food borne pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Candida albican*, and *Aspergillus niger*) were investigated. The EBN extracts were prepared using two methods: a soaking extraction method in ethyl acetate or methanol at 100-3,000 mg/L and a solvent extraction method in ethyl acetate or methanol at 20-100 mg/L. The agar well diffusion method was employed to examine antimicrobial activity of each EBN extract at various concentrations. For the soaking extraction method using methanol, the EBN extract showed stronger antimicrobial activity for *S.aureus* (100 mg/L) and *C. albican* (100 mg/L) than for *E.coli* (1,000 mg/L) and *A. niger* (>3,000 mg/L) while the EBN extract derived by the soaking extraction in ethyl acetate showed no inhibitory effects. Results of the solvent extraction method showed that the EBN extracts using ethyl acetate were more effective than using methanol with *C. albican* (20 mg/L) and *A. niger* (20 mg/L). However, using methanol showed some activity with *S. aureus* at 20-100 mg/L. Using the solvent extraction method with methanol showed slight effects with *E. coli* (>100 mg/L). This study has demonstrated a potential of the EBN extract as an antimicrobial agent against food borne pathogens.

**Keywords:** antimicrobial activity; edible bird's nest; food borne pathogens

### Introduction

The edible bird's nest (EBN) or, in Chinese, Yan Wo and Yanchao is the natural saliva nest produced by swiftlets. EBN farming is produced in many countries in Southeast Asia such as Thailand, Malaysia, Vietnam, and Indonesia. Recently, the price of EBN in Thailand is sold by swiftlet farmers at about ฿65,000 per kilogram, depending on the quality. The export value from swiftlet farming has reached around ฿126 million in 2007 (Jory and Saengthong, 2007).

It has been used as traditional Chinese medicine (Chan, 2009; Oda et al., 1998). Consumers believe that consummation of

EBN could be good for their health such as inhibition of influenza viral infection (Guo et al., 2006). This Chinese delicacy uses rare EBN in soup which makes it amongst one of the world's most expensive animal products consumed by humans. The main nutritional contents of the EBN are carbohydrates and protein with trace elements of sodium, calcium, potassium, magnesium, phosphorus, and iron (Huda et al., 2008; Marcone, 2005).

Although the nutritional aspects of the EBN have been examined, medical aspects such as antimicrobial activity have not yet been confirmed and this is the main objective of this research. Emphasis was placed on an evaluation of antimicrobial

properties of the EBN extracts against various food borne pathogens such as *Staphylococcus aureus* (gram positive bacteria), *Escherichia coli* (gram negative bacteria), *Candida albican* (yeast) and *Aspergillus niger* (mold). These microorganisms were selected because of their harm to human health. *S. aureus* is a pathogen associated with both human and animal diseases including mastitis, toxic shock syndrome, and staphylococcal food-poisoning. Symptoms can cause vomiting, abdominal cramps and diarrhea (Jørgensen et al., 2005; Hrstka et al., 2006). To date, outbreaks caused by enterohaemorrhagic *E. coli* have been attributed to strains found in foods; the vegetable, for example. Most strains of *E.coli* are harmless, but produce toxins that could cause diarrhea (Hales et al., 1991). *C. albicans* is a form of yeast that could release chemicals into the bloodstream and cause various symptoms like lethargy and chronic diarrhea (Reagan et al., 1995). *A. niger* could produce Ochratoxin A as a mycotoxin and contaminate food. It has been implicated in immunotoxicity in both animals and humans (Esteban et al., 2006).

The objective of this work is to evaluate the effectiveness of the EBN extracts to reduce four food borne pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Candida albican*, and *Aspergillus niger*) using both a soaking method and a solvent method.

## Materials and Methods

### Materials

Bird nests were collected from a local swiftlet farm in the Nakhon Si Thammarat province in Southern Thailand from June to October 2010. Feathers and dirt were removed from the nests using sterile forceps and scissors. Then, the nests were grinded in a mill to produce a fine powder and later packed into vacuum packaging and kept dry in the desiccators at 25 °C



**Figure 1** A bird's nest from a local swiftlet farm in Nakhon Si Thammarat province, southern Thailand

### Soaking Extraction Method

Milligrams (100, 500, 1,000, 2,000, and 3,000) of dried EBN were extracted and soaked in 1 L of methanol or ethyl acetate for 12 hours at 25°C in a shaker to give a concentration of 100, 500, 1,000, 2,000, and 3,000 mg/L. The extract was then filtered through a buchner funnel with Whatman No. 1 filter paper three times. Amounts of EBN's extract after filtration was between 400-500 ml. It was then preserved in a sealed vial at 4 °C until further analysis. Methanol and ethyl acetate were the control.

### Solvent Extraction Method

One hundred grams of dried EBN was soaked in 1 L of methanol or ethyl acetate, for 12 hours at 25 °C and stirred every hour using a sterilized glass rod. At the end of the extraction, it was passed through Whatman No. 1 filter paper. This filtrate was concentrated under vacuum on a rotary evaporator at 40 °C and then stored at 4 °C for further use. The crude extract was prepared by dissolving to have a stock solution of 100 mg/L concentration. Then, methanol or ethyl acetate was added onto the crude extracts to meet concentrations of 0, 20, 50, 70, and 100 mg/L.

### Microbial Strains and Culture Media

The pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli*), yeast (*Candida albicans*) and mold (*Aspergillus niger*) were obtained from the Center for Scientific and

Technology Equipment at Walailak University in Southern Thailand. Suspension of the test bacteria, yeast and mold were prepared from fully grown bacteria on Nutrient Agar, NA (Merck, Thailand) at 37°C. Fully grown yeast and mold were made on Malt Extract Agar, MEA at 25°C. Microbial strains were collected by flooding the surface of the plates with ~5 ml sterile saline solution (NaCl, 8.5 g/l water) containing Tween 80 (0.1% v/v). The viability of all strains was checked using quantitative colony counts at  $10^7$  CFU ml<sup>-1</sup>.

### Antimicrobial Activity of the EBN Extract

Antimicrobial activity of the EBN extract against *S.aureus*, *E.coli*, *C.albican* and *A. niger* were determined by the disc diffusion assay. Sterile plastic plates of 90 mm diameter (P. Intertrade Equipments, Thailand) containing NA for bacteria and MEA for yeast and mold were spread with 0.1 ml of each appropriate suspension. A cork borer was used to make a 6 mm hole on the agar. Fifty microliters of EBN extracts obtained by soaking extraction (100, 500, 1,000, 2,000, and 3,000 mg/L) and solvent extraction (20, 50, 70 and 100 mg/L) were impregnated into the hole. Fifty microliters of dilution solvent (methanol or ethyl acetate) were added to the hole on the control plates. The diameter of the zone of inhibition (mm) around the disc was measured after cultivation at 37 °C for 24 hours for bacteria and at 25°C for 72 hours for yeast and mold. The clear zone from the control plate was used to minus different clear zone from test plate.

Minimum inhibitory concentration (MIC) was tested using the broth dilution method. One ml of EBN extracts obtained by the soaking extraction (100, 500, 1,000, 2,000, and 3,000 mg/L) and the solvent extraction (20, 50, 70 and 100 mg/L) were adding 0.1 ml of in each *E.coli*, *S.aureus*, *C.albican* and *A. niger* into the sterile screw-cap tubes. Methanol and ethyl acetate were the control. The tubes were shaken

using a platform shaker at 150 rpm , 24 hours for bacteria and 72 hours for yeast and mold. The viable count of the *E.coli* and *S.aureus* in each sample was determined by plating 0.1 ml portions directly or after serially diluted in sterile 0.1% peptone water on Compact Dry "Nissui" EC (for *E. coli*), and Compact Dry "Nissui" SA (for *S. aureus*). All of Compact Drys were purchased from Oskon Ltd, Thailand. *E. coli* and *S.aureus* incubated at 37 °C for 24 hours before counting. *C. albicans* and *A. niger* were counted using MEA after incubating at 25 °C for 72 hours. The lowest concentration showing no visible growth was regarded as the MIC.

### Results and Discussion

#### Effectiveness of EBN Using the Soaking Extraction Method

Antimicrobial properties of the EBN extracts obtained by the soaking extraction method represented as a zone of inhibition is shown in Table 1. For the methanol soaking extraction, the EBN extract at 100 mg/L exhibited a clear inhibitory zone by the absence of gram positive bacterium (*S. aureus*) and yeast (*C.albican*) growth around the hole. At concentration of 1,000 mg/L, the clear zone of inhibition was observed with gram negative bacterium (*E.coli*). Growth of the test mold (*A. niger*) was, however, not affected by the presence of the EBN extract up to the concentration of 3,000 mg/L. For the ethyl acetate, no clear zone was found for all bacterial, yeast and mold test. According to the results given in Table 1, MIC of the EBN extracts weren't found at the concentration between 100 to 3,000 mg/L. These results show that soaking the EBN with methanol has a higher level of activity than ethyl acetate. Marcone (2005) reported that protein was a main composition of the EBN. In addition, it was also found that the EBN share a common 77 KDa protein that has properties similar to those of the ovotransferrin protein in eggs.

**Table 1** Effect of the EBN extract obtained by the soaking extraction on the growth of the four food-borne pathogens.

Solvent	Con. (mg/L)	Inhibition zone diameter of strains (mm.) <sup>1/</sup>			
		<i>S.aureus</i>	<i>E.coli</i>	<i>C.albican</i>	<i>A.niger</i>
Methanol	0	0±0	0±0	0±0	0±0
	100	13±1	0±0	11±4	0±0
	500	12±3	0±0	15±9	0±0
	1,000	13±4	12±3	8±3	0±0
	2,000	17±3	10±0	17±5	0±0
	3,000	11±2	10±0	8±3	0±0
	MIC <sup>2/</sup>	>3,000	>3,000	>3,000	>3,000
Ethyl acetate	0	0±0	0±0	0±0	0±0
	100	0±0	0±0	0±0	0±0
	500	0±0	0±0	0±0	0±0
	1,000	0±0	0±0	0±0	0±0
	2,000	0±0	0±0	0±0	0±0
	3,000	0±0	0±0	0±0	0±0
	MIC	>3,000	>3,000	>3,000	>3,000

<sup>1/</sup> Mean values ± standard deviations (n = 3)<sup>2/</sup> Minimum inhibitory concentration

Nevertheless, some protein could be soluble in alcohol (Mathew and Juang, 2007). These activities might depend on the compounds being extracted by methanol, the polarity of the solvents, and their intrinsic bioactivity.

### Effectiveness of EBN Using the Solvent Extraction Method

Antimicrobial properties of the EBN extracts obtained by the solvent extraction method is shown in Table 2. The EBN extract with ethyl acetate at concentrations ≥20 mg/L was capable of inhibiting the growth of yeast (*C.albicans*) and mold (*A.niger*) strains with zone inhibition of 11 to 20 mm. Although, active EBN extract from ethyl acetate showed against *S.aureus* at 70 mg/L, it could not inhibit *E.coli* in this test at concentration of 100 mg/L. The methanol extract of the EBN also showed effect on *S.aureus* at 20 mg/L and slightly effect on *E.coil* at 100 mg/L, but did not show any anti yeast and mold. It may be due to low concentration of EBN. Therefore, our next study will focus on different higher concentration of the EBN.

**Table 2** Effect of the EBN extract obtained by solvent extraction on the growth of the four food-borne pathogens.

Solvent	Con. (mg/L)	Inhibition zone diameter of strains (mm.) <sup>1/</sup>			
		<i>S.aureus</i>	<i>E.coli</i>	<i>C.albican</i>	<i>A.niger</i>
Methanol	0	0±0	0±0	0±0	0±0
	20	10±0	0±0	0±0	0±0
	50	8±1	0±0	0±0	0±0
	70	8±1	0±0	0±0	0±0
	100	9±0	4±0	0±0	0±0
	MIC <sup>2/</sup>	>100	>100	>100	>100
Ethyl acetate	0	0±0	0±0	0±0	0±0
	20	0±0	0±0	14±2	11±3
	50	0±0	0±0	14±4	11±0
	70	13±1	0±0	20±4	11±3
	100	10±0	0±0	20±0	11±0
	MIC	>100	>100	>100	>100

<sup>1/</sup> Mean values ± standard deviations (n = 3)<sup>2/</sup> Minimum inhibitory concentration

The crude extracts with MIC of the EBN in all samples were more than 100 mg/L. The relationship between the zone of inhibition and MIC value may not be related. On the other hand, these test strains may have different level of intrinsic tolerance to antimicrobials and thus the MIC values differ from isolate to isolate. There are limited reports on the antimicrobial activity of the EBN extract in the literature, even though inhibition of some components in the EBN extract have been reported against viruses (Guo et al., 2006), *Staphylococcus aureus*, *Streptococcus* sp., *Escherichia coli*, *Salmonella* sp., *Klebsiella pneumonia*, and *Pasteurella multocida* (Suriya, et al., 2004). However, in depth exploration on antibacterial activity of the bird's nest needs to be carried out. The future work should be focused on determination of major active components in the EBN extract responsible for its antimicrobial activity against food borne pathogens.

### Conclusions

The EBN extract obtained by soaking the extraction in methanol showed good inhibition against growth of gram positive bacteria and yeast. The EBN extract obtained by solvent extraction using ethyl

acetate at concentration  $\geq 20$  mg/L inhibited growth of yeast and mold (*C. albicans* and *A. niger*). The minimal inhibitory concentration of the EBN were not found for all samples in this test. The EBN extract showed good potential as a healthy antimicrobial agent against food borne pathogens in the alimentary canal.

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### References

- Chan, S.W. 2009. Review of Scientific Research on Edible Bird's Nest [online] available: <http://www.hkfsta.com.hk/articles/special/article7.htm>. Accessed in October, 2009.
- Esteban, A., Abarca, M.L., Bragulat, M.R. and Cabañes F.J. 2006. Effect of water activity on ochratoxin A production by *Aspergillus niger* aggregate species. *Int. J. Food Microbiol.* 108(2): 188-195.
- Guo, C-T., Takahashi, T., Bukawa, W., Takahashi, N., Yagi, H., Kato, K., Hidari, K., I-P. Jwa., Miyamoto, D., Suzuki, T. and Suzuki, Y. 2006. Edible bird's nest extract inhibits influenza virus infection. *Antivir Res.* 70: 140-146.
- Hales, B.A., Fletcher, J.N., Ridha, G., Batt, R.M., Hart, C.A. and Saunders, J.R. 1991. Incidence of common DNA sequences in bovine and porcine *Escherichia coli* strains causing diarrhea. *Res Vet Sci.* 50(3): 355-357.
- Hrstka, R., Růžicková, V., Petráš, P., Pantůček, R., Rosypal, S. and Doškar, J. 2006. Genotypic characterization of toxic shock syndrome toxin-1-producing strains of *Staphylococcus aureus* isolated in the Czech Republic. *Int J Med Microbiol.* 296(1): 49-54.
- Huda, N-M.Z., Zuki, A.B.Z., Azhar, K., Goh, Y.M., Suhaimi, H., Hazmai, A-A.J. and Zairi, M.S. 2008. Proximate, Elemental and Fatty Acid Analysis of Pre-Processed Edible Bird's Nest (*Aerodramus fuciphagus*) : A Comparison Between Regions And Type of nest. *J Food Tech.* 6: 39-44.
- Jørgensen, H.J., Mathisen, T., Løvseth, A., Omoe, K., Qvale, K.S. and Loncarevic, S. 2005. An outbreak of Staphylococcal food poisoning caused by enterotoxin H in mashed potato made with raw milk. *FEMS Microbiol Lett.* 252(2): 267-272.
- Jory, P. and Saengthong, J. (2007). *Birds' nests: secrets of a billion-dollar business*. Nakhon Si Thammarat, Thailand Research Fund; Regional Studies Program, Walailak University.
- Marcone, M.F. 2005. Characterization of the edible bird's nest the "Caviar of the East". *Food Res Int.* 38: 1125-1134.
- Mathew, D.S. and Juang, R-S. 2007. Role of alcohols in the formation of inverse microemulsions and back extraction of proteins/enzymes in a reverse micellar system. *Sep Purif Technol.* 53(3): 199-215.
- Oda, M., Ohta, S., Suga, T. and Aoki, T. 1998. Study on Food Components: The Structure of N-Linked Asialo Carbohydrate from the Edible Bird's Nest Built by *Collocalia fuciphaga*. *J Agr Food Chem.* 46: 3047-3053.
- Reagan, D.R., Pfaller, M.A., Hollis, R.J. and Wenzel, R.P. 1995. Evidence of nosocomial spread of *Candida albicans* causing bloodstream infection in a neonatal intensive care unit. *Diagn Micr Infec Dis.* 21(4): 191-194.
- Suriya R. et al. 2004. Preliminary in-vitro study on antibacterial activity of swiftlet bird's nests. In: *Animal health: a breakpoint in economic development? The 11th International Conference of the Association of Institutions for Tropical Veterinary Medicine and 16th Veterinary Association Malaysia Congress, 23-27 August 2004, Petaling Jaya, Malaysia.*

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