Antimicrobial Effect of *Centella Asiatica* Extract On Fish Surimi

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Abstract

Centella asiatica or commonly known as Centella is a herb that is often used by everyone in the world for health reasons. Centella usually used to cure skin diseases such as leprosy, scabies, ulcers and wounds. The antimicrobial properties of Centella or locally known as "Pegaga" has been studied in many previous research especially in medicinal benefits. There were some scientific studies showed that Centella can also functioning as an antimicrobial in food. Therefore, this study was to determine antimicrobial effects of Centella extract on food, specifically on fish surimi. The rational of using fish surimi was it is a perishable food that requires food preservative to prolong it shelflife. Centella has been extracted by using methanol as a solvent. There were two methods of adding extract to fish surimi. Centella extract was added in sample or has been dropped onto media that has been spreader with sample. Total Plate Count Method was used to determine microbial numbers in fish surimi that have been added with C. asiatica extract meanwhile Disc Diffusion Method was used to determine antimirobial effects of Centella extract by measuring the inhibitory zone on media used. Different concentrations of Centella extract were used. 10%, 30%, 50%, 70% and 90% of extract have been tested on fish surimi. Based on results obtained, it showed that the total microbial numbers in fish surimi was reducing when the concentration of extract is increased. Meanwhile the inhibition zone was increasing when the concentration of extract is increased. It proved that Centella extract can inhibit microbial growth on fish surimi. It means that Centella has an antimicrobial effect on fish surimi.

Keywords: Antimicrobial, Centella Extract, Fish Surimi

1. Introduction

1.1 Centella asiatica

Centella asiatica or commonly known as Centella is a herb that is often used by everyone in the world for health reasons. It is also known as the Asiatic pennywort or Indian pennywort in English, among various other names in other languages (Gohil et. al., 2010). Centella usually used to cure among various other names in other languages (In 1949 a study was conducted on the

herbs and found that it is potent cure leprosy and ulcers because of its antibacterial properties (Goh et. al., 1995). The research conducted in 1950 found that the herbs are very good for healing wounds (Kimura et. al., 2008), sores and skin irritation cure.
The Chinese society depends on the herbs for treating emotional disorders such as emotional stress caused by physical problems. *Centella* has been recognized by the World Health Organization (WHO) as a herb that generate intelligence to think, especially among children. WHO recognizes that the herbs are rich in various medicinal ingredients. This herb is very effective and will improve the functioning of the mind such as improving memory and concentration of mind (Subathra et al., 2005).

The use of *Centella* in food and beverages has increased over the years basically due to its health benefits such as antioxidant (Hamid et al., 2002; Vimala et al., 2003). There were some scientific studies that have been conducted; it appears that *Centella* can also functioning as an antimicrobial in food. The study shows that there is inhibition of the activity of methanol extracts obtained from Centella (Junofy, 2013). *Centella* has been tested on some microorganisms such as E. Coli andStaphylococcus *aureus* (Dora et. al., 2012).

1.2 Fish Surimi
Surimi is a paste made from fish or other meat, as well as multiple Asian foods that use surimi as its primary ingredient. It is available in many shapes, forms, and textures, and often used to mimic the texture and color of the meat of lobster, crab, and other shellfish (Park, 2000). Surimi is prepared from the mechanically deboned, washed (bleached) and stabilized flesh of fish. It is an intermediate product used in the preparation of a variety of ready to eat seafood such as kamaboko, fish sausage, crab legs and imitation shrimp products.

Surimi-based products are gaining more prominence worldwide, because of the emergence of Japanese restaurants and culinary traditions in North America, Europe and elsewhere. Ideally, surimi should be made from low-value, white fish with excellent gelling ability and which are abundant and available year-round. At present, Alaskan pollack accounts for a large proportion of the surimi supply. Other species, such as sardine, mackerel, barracuda, striped mullet have been successfully used for surimi production (Hall, 1997).

Fish surimi is a lean meat from fish that has been separated or minced. The meat then is rinsed numerous times to eliminate undesirable odors. The result is beaten and pulverized to form a gelatinous paste. Depending on the desired texture and flavor of the surimi product, the gelatinous paste is mixed with differing proportions of additives such as starch, egg white, salt, vegetable oil, humectants, sorbitol, sugar, soy protein, seasonings, and enhancers such as

transglutaminases and monosodium glutamate (MSG). If the surimi is to be packed and frozen, food-grade cryoprotectants are added as preservatives while the meat paste is being mixed (Park et. al., 2007). Under most circumstances, surimi is processed immediately into a formed and cured product. According to the United States Department of Agriculture National Nutrient Database, fish surimi contains about 76% water, 15% protein, 6.85% carbohydrate, and 0.9% fat.

2. Problem Statement

Fish is a highly perishable food which needs proper handling and preservation if it is to have a long shelf life and retain a desirable quality and nutritional value (Hall G.M, 1997). The central concern of fish processing is to prevent fish from deteriorating. The most obvious method for preserving the quality of fish is to keep them alive until they are ready for cooking and eating. Surimi is an example of fish products. Surimi is also a highly perishable food and needs preservatives, pasteurization process and cold temperature to delay spoilage. Sorbitol is an example of preservatives used to avoid microbial spoilage in surimi (Celeste, 2014)

However, the growing demands of health-conscious consumers on food products of high quality, all-natural, wholesome, minimally processed, free of synthetic chemical ingredients, long storage life and also limiting the use of preservatives permitted in various foods leads to this study which is to find a natural alternative preservative agent -based plant and guaranteed safe for use in food processing industry. The plant is less exploited resources in the pursuit of alternative antimicrobial agent which structures and reactions may vary. Use a preservative agent overdose is suspected hazardous chemical as toxicity. Therefore, the food industry urged to either completely eliminate chemical preservative agent from the product or find an alternative that is natural to extend the storage life of food products.

Information regarding antimicrobial activity of Malaysian herbal extracts (such as extracts are effective, the concentration level of effectiveness, the type of microorganisms and the resistance and effective) can be documented for communication with scientists around the world and to reference studies to be carried out. According to Mitcher (1975), the plants are less exploited resources in the pursuit of alternative antimicrobial agents in the structure and mode of action may be very different from antimicrobial agent found from sources known. So, Malaysia herbs has great potential for research in the quest for alternative antimicrobial agents that might be better than a synthetic antimicrobial agent that is widely used now. Despite all these, Centella asiatica is used to determine its antimicrobial effects on fish surimi.

3. Objectives

To determine the number of microorganisms in fish surimi added with *Centella* extract, to observe the inhibition effects of *Centella* extract onmicrobial growth in fish surimi and to determine the differences of inhibition zone of *Centella* extract with 10%, 30%, 50%, 70% and 90% concentration.

4. Methods and Materials

4.1 Extraction Method

Centella leaves were removed from stalk. Then it has been dried in anoven at a temperature of 60 ° C for 1 hour. After cleaning, the leaves finely milled to a powder form. 15 gram powder was mix with 500ml of methanol. The mixture was placed in to the "water bath" for 8 hours so that a solution was quickly dissolved. The solution was extracted using rotavap machine (rotary evaporation) until it produce a concentrated extract (Duangkamol et. al., 2008).

4.2 Serial Dilution

Serial dilution is a repeated dilution performed on a sample of the fluid in order to get an average of the highest dilution volume of maneuverability. This method is performed to remove a small volume serial number in sequence. Serial dilution is basically reducing a concentration by the addition of dilution factor equal proportion. 10g of sample (fish surimi) is homogenized. 90ml peptone water is added to make the 1:10 dilution or 10^{-1}. Then 1 ml of 1:4 dilution was added into 9 ml of peptone water in the bottle until the second dilution is achieved by 6 dilution. Pipette used should be sterilized and changed for each transfer as to avoid contamination during the experiment (Wallace et. al., 1998).

4.3 Total Plate Count Method

Total plate count method, or alternatively the TPC is to determine microbial numbers in sample which is fish surimi that was mixed with *Centella* extract. 1 ml Sample was transferred into petri dishes thatcontain Nutrient Agar. The sample was spread using a hockey stick. Then, the petri dishes were incubated in 37°C incubator for 24-48 hours. Colony growth on Nutrient Agar was count and calculated (Larry et. al., 1998).

4.4 Disc Diffusion Method

Disc diffusion method, or alternatively (Kirby Bauer Test) is disc containing antimicrobial agents (*Centella* extract) with 10%, 30%, 50%, 70% and 90% concentration were put on the surface of agar plates which contained media inoculated with sample. Media used is Nutrient Agar which is suitable for all microorganisms. Antimicrobial agent was diffused into the media, inhibit microorganisms in which antimicrobial agents are inoculated, depending on how sensitive antimicrobial material. The petri dishes were then incubated in 37°C incubator for 24-48 hours. Observation on inhibition zone was done when the period completed.

5. Results and Discussion

5.1 Effect of Centella extract on total microbial growth in sample.

Table 1. Numbers of microbial growth on Nutrient Agar

Percentage of Extract	Total Number of Microorganism
Standard (0%)	2.745×10^7 CFU/g ± 2.341×10^7
10 %	3.585×10^6 CFU/g ± 4.048×10^6
30 %	4.23×10^5 CFU/g ± 2.857×10^5
50 %	4.815×10^4 CFU/g ± 4.716×10^4
70 %	2.498×10^4 CFU/g) ± 2.196×10^4
90 %	2.365×10^4 CFU/g ± 2.383×10^4

Centella extract was proved that can reduce microbial numbers. Based on TPC analysis, microbial numbers decreases as the *Centella* extract is added compared to the standard which is fish surimi with no extract. Refer to Table 1, it showed that number of miroorganism is decreases as the concentration of *Centella* extract is increase. The lowest number of microbial growth is observed in the 90% *Centella* extract. According to Junofy et. al. (2013), Centella extract can also act as effective antimicrobial agents that can be used alone or in combination in medicines or as natural food preservatives to retain the quality of food and prevent its spoilage. Through the scope of the study, it found that the role of *Centella* has antimirobial properties in food products. It can inhibit microorganism growth in food.

5.2 Effect of Centella Extract on inhibition zone

Table 2. The diameter of zone of inhibition

Percentage of C. Asiatica extract	The diameter of zone of inhibition	
Standard (0%)	0 mm	
10 %	2.25 mm ± 3.54	
30 %	3.25 mm ± 3.54	
50 %	3.5 mm ± 0	
70 %	5.75 mm ± 1.061	
90 %	13.75 mm ± 1.768	

The diameter of the zone of inhibition of the growth were measured by the use of the scale ruler in milliliter (mm) clear zones of inhibition indicated the susceptibility of the organism to the extracts while absence of such zones showed resistance or no inhibitory effect of extracts on the microorganism. Nutrient Agar (NA) was used as a growth medium of microorganisms designed

to observe inhibitory effect of *Centella* extract. The methabolic extract of Centella asiatica shows significant antimicrobial activity against microorganisms as assessed by the diameter of zone of inhibition. The highest diameter of zone of inhibition is on 90% of *Centella* extracts. The inhibitory effects determined by the zone of inhibition increases with increase in concentration of extracts. This confirmed the report by Dora et. al., (2012), who reported that Centella asiatica leaves showed that the leaves contain some bioactive components that have some inhibitory effects on some microorganisms.

6. Conclusion

The findings in the study showed the inhibitory effects of *Centellaasiatica* against microorganisms in fish surimi is increases when the concentrations of extracts are increase while the number of microorganism is decreases.

References

Brinkhaus, B., Lindner, M., Schuppan, D. and Hahn, E.G. (2000). Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella asiatica*. Phytomedicine. 7: 427-428.

Celeste, H. (2014). *What is surimi?* Conjecture Corporation. Dalton, Louisa (2002). "Food Preservatives". *Chemical andEngineering News.* 80 (45): 40.

Dora, I., U., Naomi, U., A., Danladi, N., B., and Otung, E. (2012). *Inhibitory Effect of Varying Concentrations of Leaves' Extracts of Centella Asiatica* (Gotu Kola) on Some Microorganisms of Medical Importance. Vol. 3, No. 2, 142-148.

Duangkamol T., Theera R., Thavajchai S., Nongluck R., (2008). A Study on Antibacterial Activity of Crude Extracts of Asiatic Pennywort and Water Pennywort Against *Staphylococcus aureus.*

Goh, S.H., Chuah, C.H., Mok, J.S.L., Soepadmo, E. (1995). Malaysian medicinal plants for the treatment of cardiovascular diseases. *Pelanduk,* Malaysia, 63.

Gohil KJ, Patel J.A, Gajjar A.K. (2010). Pharmacological Review on Centella asiatica: A Potential Herbal Cure-all. *Indian J Pharm Sci.* 2010 Sep;72(5):546-56.

Hall, G., M.(1997). Fish processing technology Springer. ISBN 978-0-7514-0273-5.

Hamid, A. A, Md Shah, Z., Muse, R. and Mohamed, S. (2002). Characterization of antioxidative of *Centella asiatica* (L) Urban, *Food Chemistry* 77: 465-469.

Junofy Anto Rozarina. N, (2013). Antimicrobial Potentials of The Methanolic Extracts of Plants. *IJSRR* 2013, 2(1) Suppl., 89-95.

K. Seetaramaiah, A. Anton Smith, R. Murali, R. Manavalan. (2011). *Preservatives in Food Products*.

Kimura, Y., Sumiyoshi, M., Samukawa, K., Satake N. and Sakanaka M., (2008). Facilitating action of asiaticoside at low doses on burn wound repair and its mechanism. *European Journal of Pharmacology*. 584: 415-421.

Larry M., James T. P., (1998). *Bacteriological Analytical Manual*, Edition 8, Revision A, Chapter 3. Aerobic Plate Count.

Nychas G.J.E. (1995). *Natural antimicrobials from plants*. In: Gould GW (Ed) New Methods of Food Preservation, Blackie Academic Professional, London, pp 58-89.

Park, J., W.(2005). Surimi and Surimi Seafood. Second Ed. Park, J., W. editor. CRC Press. 960.

Park, J., W., Lanier, T. C. (2000). *Processing of surimi and surimi seafoods*. In: Martin RE, Carter EP, Flick Jr. GJ, Davis LM, editors. Marine and freshwater products handbook. Technomic Publishing Co.Lancaster. 417-430.

Subathra, M., Shila, S., Devi, M. A. and Panneerselvam, C. (2005). Emerging role of Centella asiatica in improving age-related neurological antioxidant status. *Experimental Gerontology* 40: 707-715.

Vimala, S., Adenan, M. I., Ahmad, A. R. and Shahdan, R. (2003). *Nature's choice to wellness: antioxidant vegetables/ulam*. Siri Alam dan Rimba No.7. Kuala Lumpur: FRIM.p. 90-92.

Wallace H. A., Thomas S. H., (1998). *Bacteriological Analytical Manual*, Edition 8, Revision A, Chapter 1 Food Sampling and Preparation of Sample Homogenate.