



Antibacterial effect of ethanol extract of nutmeg against foodborne disease pathogens



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ABSTRACT

Foodborne disease is a disease that occurs as a result of food contamination by microbes or harmful chemicals. Nutmeg (*Myristica fragrans* Houtt.) is widely used as a spice and flavor enhancer for food and beverages. In addition, it has traditionally been used to treat diarrhea and kidney disease. Several pharmacological studies have reported the antioxidant, antimicrobial, antidiarrheal and anti-inflammatory activity of this plant. Antibacterial effectiveness test of nutmeg against several pathogens in this study was carried out using the disc diffusion method. The results of this study indicated that the ethanol extract of flesh of fruit (concentration of 5%, 10%, 15%, 20%, and 25%) had antibacterial activity against all tested pathogenic bacteria. Different results were shown by the ethanol extract of seed and mace. All the ethanol extract concentrations of seed were not able to inhibit the growth of *Shigella sp.* and *Clostridium sp.*, while the ethanol extract of mace in all concentrations was unable to inhibit the growth of *Shigella sp.* Various factors may affecting this extract and compound activity when used in complex biological systems such as in vivo and human studies. Future studies must also focus on aspects of pharmacokinetics and toxicological plant extracts and phytochemicals.

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INTRODUCTION

Foodborne illness is defined by the World Health Organization (WHO) as Diseases, usually infectious or toxic in nature, caused by agents that enter the body through food consumption. WHO estimates that foodborne and waterborne (water) transmitted diarrhea around the world

together, can kill an estimated 2.2 million people each year (WHO, 2015). Foodborne disease is a disease that occurs as a result of food contamination by microbes or harmful chemicals. Microbes have a virulence factor that allows them to infect humans. Therefore, foodborne disease by microbes is more dangerous (Kassem, 2018). Bacteria and fungi are the most common microorganisms as the main causes of foodborne disease. In developing countries such as Indonesia, where the facilities and infrastructure for prevention efforts are still inadequate, more than 150 million cases of foodborne disease due to microbes have been reported (Nadiya & Asharina, 2016).

Foodborne disease continue to receive attention, food safety researchers and policy makers to pay more attention to the number of outbreaks of disease which continues to rise due to some pathogenic and spoilage microorganisms in food (Shonhiwa, Ntshoe, Essel, Thomas, & Mccarthy, 2017). Foodborne disease has been linked to the consumption of foods such as poultry, beef, processed meat, cheese, seafood and other types of food. Food can be contaminated through raw materials, cross-contamination and poor sanitary behavior. The increasing antibiotic resistance of several foodborne disease pathogens has generated interest in exploring new antimicrobials (Anibijuwon & Omojasola, 2013). The antimicrobial properties associated with some spices are believed efficacious. It is estimated that society has used about 10% of all plants on earth to treat various infections, although only 1% has been recognized by modern scientists (Lewis & Ausubel, 2006). Medicinal plant-based antimicrobials are an untapped source of medicines. Scientific clarification of these claims is needed to gain a better understanding of the use of this spice in infection and disease management, as well as in overcoming the problem of antibiotics (Lewis & Ausubel, 2006).

Nutmeg, *Myristica fragrans* Houtt. (family: Myristicaceae) is a type of plant originating from Maluku Indonesia. However, it has been successfully cultivated in other Asian countries, such as India, Malaysia, Sri Lanka, and Caribbean islands, especially in Grenada and Trinidad. This aromatic plant can grow as high as 9-12 m with spreading branches and yellow-fleshed fruit. Inside the ripe fruit, there are brown seeds and red, fleshy seeds covering the kernels (Simamora, Santoso, & Timotius, 2018). Nutmeg is widely used as a spice and flavor enhancer for food and beverages. In addition, it has traditionally been used to treat diarrhea and kidney disease. Several pharmacological studies have reported the antioxidant, antimicrobial, antidiarrheal and anti-inflammatory activity of this plant. However, most of the previous research has focused only on essential oils or organic extracts from the seeds and mace parts of Nutmeg, and has not been comprehensively researched.

RESEARCH METHODS

Research Design

This research is a laboratory experimental study, using a true experimental design with a post test only control group design. The posttest-only control group design is a research design in which there are at least two groups, one of which does not receive a treatment or intervention, and data are collected on the outcome measure after the treatment or intervention.

Population and Samples

Nutmeg were bought from local market, Ambon, Maluku, Indonesia. The botanical identification of nutmeg was confirmed at the Biology Department, Faculty of Mathematics and Natural Sciences, Pattimura University by comparison of the fresh material to their dried, pressed plant materials stored in the herbarium collection.

The tested pathogenic bacteria is a collection of the microbiology laboratory, Biology Department, Science and Technology Faculty, UIN Alauddin Makassar. *E. coli*, *Salmonella typhi*,

Vibrio cholerae, *Shigella* sp., and *Clostridium* sp. regenerated into 5 mL NB media (Nutrient Broth) then incubated at 28-30°C for 24 hours.

Procedures

Preparation of ethanol extract of Nutmeg (*Myristica fragrans* Houtt.)

The nutmegs obtained were separated between the flesh of fruit, mace and seed, cleaned and cut into small pieces, then dried and aerating for 3 days. Furthermore, each of the flesh of fruit, mace and seed is blended until smooth. The blended flesh of fruit, mace and seed were then weighed as much as 1,000 grams each and soaked in 1,000 ml each for 24 hours. Then the ethanol mixture is filtered to separate the filtrate and residue. The filtrate obtained which contains a lot of solvent must be concentrated by a rotary evaporator at a temperature of 45 ° C. The result of this concentration is called an extract. The resulting extract will be made into multiple concentrations of 5%, 10%, 15%, 20% and 25%.

Antibacterial assay

This antibacterial assay using Disk Paper Diffusion Method of Kirby Bauer . The bacterial suspension was spread using sterile cotton swabs over the MHA (Mueller Hinton Agar) media, the petri dish was rotated at an angle of 60° until the bacterial suspension was evenly distributed on the surface of the MHA. The empty disc paper was dripped using a micro pipette as much as 20 µl of the nutmeg extract. Aquades as a negative control (K-) and paper discs of chloramphenicol as a positive control (K +) were placed on MHA media that had been inoculated by the test bacteria. Results incubation for 24 hours was observed inhibition zone is formed on the growth of test bacteria and measured the diameter of inhibition zone formed.

Data Analysis

Processing and data analysis descriptively with observation of diameter clear zone. Data is expressed as mean plus or minus standard deviation (mean \pm SD).

RESULTS

Antibacterial effectiveness test against several pathogens in this study was carried out using the disc diffusion method. The results of this study indicated that the ethanol extract of flesh of fruit (concentration of 5%, 10%, 15%, 20%, and 25%) had antibacterial activity against all tested pathogenic bacteria (Table I). Different results were shown by the ethanol extract of seed and mace. All the ethanol extract concentrations of seed were not able to inhibit the growth of *Shigella* sp. and *Clostridium* sp., while the ethanol extract of mace in all concentrations was unable to inhibit the growth of *Shigella* sp.

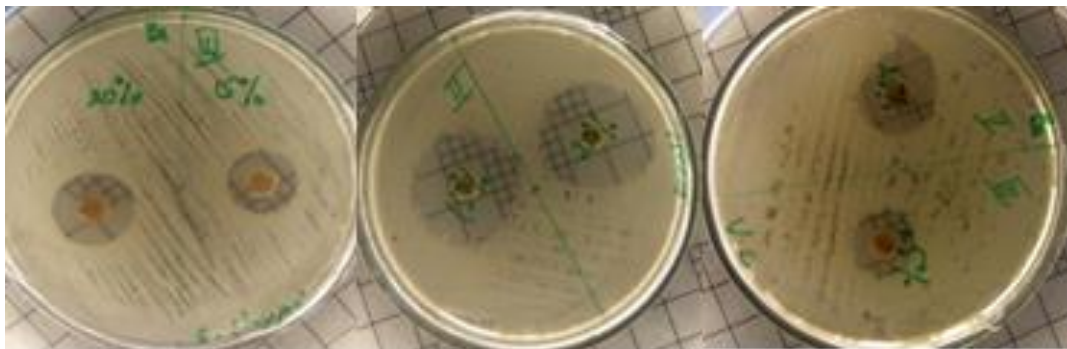


Figure 1. The clear zone formed in the inhibition test of the ethanol extract of nutmeg against pathogens.

Table I. Inhibition test of the ethanol extract of nutmeg against pathogenic bacteria

Extract	Concentration	inhibition zone (cm)				
		<i>S.thypi</i>	<i>E. coli</i>	<i>V. cholera</i>	<i>Shigella</i> sp.	<i>Clostridium</i> sp.
Flesh of fruit	5%	0,8 ± 0	2,1 ± 0,06	0,7 ± 0,61	0,9 ± 0,10	0,8 ± 0,06
	10%	0,9 ± 0,06	2,3 ± 0,06	1,2 ± 0,25	1,1 ± 0,12	1,1 ± 0,21
	15%	1,2 ± 0,06	2,0 ± 0,44	1,2 ± 0,21	1,3 ± 0,21	1,0 ± 0,06
	20%	1,4 ± 0,15	2,3 ± 0,21	1,4 ± 0,06	1,5 ± 0,17	1,1 ± 0,23
	25%	1,5 ± 0,17	2,2 ± 0,29	1,5 ± 0,10	1,4 ± 0,23	1,2 ± 0,12
Seed	5%	1,0 ± 0,14	2,1 ± 0	0,3 ± 0,46	0	0
	10%	1,1 ± 0,06	2,2 ± 0,12	1,0 ± 0,12	0	0
	15%	1,2 ± 0,06	2,1 ± 0,1	1,5 ± 0,25	0	0
	20%	1,2 ± 0,15	2,0 ± 0,21	1,5 ± 0,06	0	0
	25%	1,2 ± 0,26	2,0 ± 0,66	1,6 ± 0,40	0	0
Mace	5%	1,5 ± 0	0,9 ± 0,32	2,0 ± 0,26	0	0,6 ± 0,49
	10%	1,4 ± 0,06	1,3 ± 0,26	2,2 ± 0,06	0	0,5 ± 0,52
	15%	1,2 ± 0,17	1,0 ± 0,06	1,8 ± 0,36	0	1,0 ± 0,55
	20%	1,2 ± 0,21	1,1 ± 0,17	1,8 ± 0,06	0	0,3 ± 0,58
	25%	1,4 ± 0,30	1,2 ± 0,10	2,0 ± 0,36	0	0,6 ± 0,06

DISCUSSION

Although spices have been widely used in rituals, and as flavoring and decorating agencies since ancient times, the recent literature has increasingly reported the antibacterial activity of spices against gram positive bacteria and grams negative responsible for human infectious diseases and food safety problems. Examples of spices are cinnamon, oregano, nutmeg, basil, pepper, thyme, clove, rosemary, ginger, cumin, etc. However, several studies focused on the inhibition effects of these spices in antibiotic-resistant bacteria (Zhang et al., 2019). Nutmeg (*Myristica fragrans* Houtt.) is highly applied in the food sector Spices and aromatic plants, because of the bioactive components, such as phenol, carotene, flavonoids, and vitamins (Kiarsi, Hojjati, Behbahani, & Noshad, 2020).

The formation of the clear zone (figure 1) indicates inhibition and indicates the presence of antibacterial compounds that can inhibit the growth of pathogenic bacteria. The results of previous studies have stated that high levels of flavonoids are found in turmeric, ginger and nutmeg. The substance content in the flesh of fruit of 100g nutmeg contains approximately 10g of water, 7g protein, 33g fat, volatile oil with the main components of hydrocarbon monoterpenes such as pinen, sabinene, monoterpenic acids, and aromatic ether such as myristicin and elimin (Rahadian, 2009). The flesh of fruit of nutmeg contains aromatic flavor substances consisting of essential oils, namely myristicin and monoterpenes and the myristicin content in the flesh of the nutmeg based on the research results of Rahadian (2009), which is higher than the essential oils from seeds and mace. Antibacterial activity of this phenolic compound involves many action modes, such as destroying cell membrane morphology, changing membrane fatty acids, thinning the style of proton motif, causing reactive oxygen damage, damaging the enzymatic mechanism for energy and metabolic production, disrupting normal protein functionality, and inhibits nucleic acid (Lal, Chandraker, & Shukla, 2020; Shan, Cai, Brooks, & Corke, 2007).

Antibacterial efficacy for gram positive and gram negative organisms indicate that nutmeg can be used for broad spectrum antibiotics. (Shafiei, Shuhairi, Md Fazly Shah Yap, Harry

Sibungkil, & Latip, 2012). Like others solvent extracts of nutmeg that have been variously reported to have antibacterial activity, water extract of nutmeg also have antimicrobial effects. (Nurjanah, Putri, & Sugiarti, 2018) reported that essential oil of nutmeg have antimicrobial effects against gram positive bacteria such as *Staphylococcus epidermis*, *Staphylococcus aureus* and gram negative bacteria such as *Salmonella typhi*, *Shigella dysenteriae*. Nurhasanah (2014) reported that methanol fruit extracts of nutmeg is potent against *Escherichia coli* and *Staphylococcus aureus*. Gupta, Bansal, Babu, & Maithil (2013) reported that acetone, ethanol, methanol, butanol and water extract of nutmeg have potent effect against some species of fungi (*Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus flavus*), gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) and negative bacteria (*Pseudomonas putida*, *Pseudomonas aeruginosa*). Ibrahim, Naem, & Abd-Sahib (2013) reported that ethanol and acetone seed extract of nutmeg is potent against gram positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis*, while ethanol, acetone seed extracts is not potent against gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The authors further reported that water extract is not potent against both gram positive and negative bacteria. The difference in the efficacy of different study could be due to the age of the plant, environmental condition that the plant was cultivated, concentration of the extract used for the sensitivity testing, and choice of solvent (Izah, Zige, Alagoa, Uahunmwangho, & Iyamu, 2018).

Antibacterial activity seems to depend on bacteria, and gram positive bacteria are more susceptible to extract spices tested than gram negative bacteria, which are in accordance with many previous studies (Ali, Hamiduddin, Zaigham, & Ikram, 2018; Benmeziane, Djermoune-Arkoub, Adamou Hassan, & Zeghad, 2018). In contrast to gram positive bacteria, gram negative bacteria have outer membranes that are rich in lipopolysaccharides, as well as unique periplasmic spaces. Complex compositions and spatial structures of lipopolysaccharides form a barrier to the penetration of antimicrobial agents, in addition, the existence of enzymes in the periplasmic space can break down intrusive molecules, prevent antibacterial drugs to enter the intracellular environment (Shan et al., 2007).

CONCLUSION

Nutmeg (*Myristica fragrans* Houtt.) has emerged as a credible new antimicrobial source. This study evaluates the efficacy of antibacterial ethanol extract from nutmeg. This study shows that the nutmeg has antibacterial activity against gram positive and negative bacteria. Thus, it can be used for broad spectrum antibiotics. Various factors may affecting this extract and compound activity when used in complex biological systems such as in vivo and human studies. Future studies must also focus on aspects of pharmacokinetics and toxicological plant extracts and phytochemicals.

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