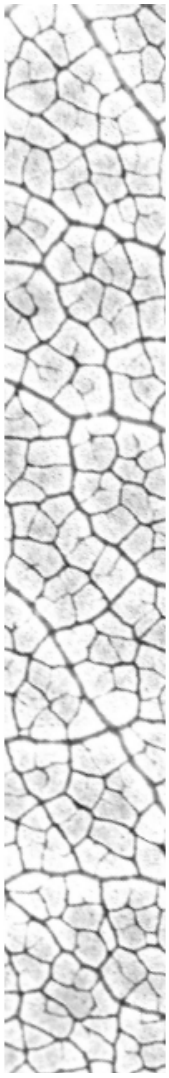

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The antibacterial and antifungal activity of essential oil derived from the flesh of nutmeg fruit

Sophia Grace Sipahelut [1], John Alfred Patty [2], Zeth Patty [3], Ariance Yeane Kastanja [3], Vanessa Natalie Jane Lekahena [4*]

[1] Agricultural Product Technology, Faculty of Agriculture, Pattimura University, Ambon, Maluku, INDONESIA
[2] Agroecotechnology, Agricultural Cultivation, Faculty of Agriculture, Pattimura University, Ambon, Maluku, INDONESIA
[3] Faculty of Science, Technology and Health, Hein Namotemo University, Tobelo, Maluku Utara, INDONESIA
[4] Fishery Product Technology Study Program Faculty of Agriculture Universitas Muhammadiyah Maluku Utara, Ternate, Maluku Utara, INDONESIA
*Corresponding author: enchalekahena@ummu.ac.id

Abstract
This research aims at determining the antibacterial and antifungal activity of the Nutmeg fruit flesh-derived oil with some concentration ratios of nutmeg fruit flesh-derived oil to ethanol (1:0, 1:1, 1:2 and 1:3) and examining the essential oil's inhibiting activity of the growth of pathogenic bacteria, food-destroying bacteria and food-destroying fungi. The bacteria tested include *Staphylococcus aureus* and *Escherichia coli*, whereas the fungi tested are *Aspergillus flavus* and *Fusarium moniliforme*. This study employs the agar diffusion and total plate count methods. The results show that the nutmeg fruit flesh-derived oil may inhibit the growth of isolates at all concentrations. The higher of the concentration of nutmeg fruit flesh-derived oil, the wider the bacterial growth inhibition zone is. The utilization of nutmeg fruit flesh-derived oil at all concentrations shows strong antibacterial power category against all tested bacteria. However, it shows weak antifungal power against all tested fungi.

Keywords: nutmeg fruit flesh-derived oil, antibacterial activity, antifungal activity

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INTRODUCTION

Infectious diseases are the main cause of death both in advanced and developing countries (Lewis *et al.* 2006). About half of deaths in tropical countries are caused by bacterial infection (Ayoola *et al.* 2008, Iwu *et al.* 1999). Antibiotic invention and development have successfully cured various infectious diseases. However, the antibacterial substance development is accompanied with arising resistance to drugs because of irrationally frequent uses of antibiotics (Ayoola *et al.* 2008).

Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella spp, Staphylococcus koagulase-negatif, Shigella, Enterococcus sp. and *Escherichia coli* are some of the main bacteria with multidrug resistance (Chouhan *et al*. 2017) which cause people's worry about microbial resistance to conventional preservatives (Schuenzel and Harrison 2002). This condition makes demand for safe, healthy and natural foods increase since the people are concerned about chemical residue existing in the foods they consume (Charu Gupta *et al.* 2008).

Consumers' high demand of foods free from synthetic chemical compounds causes development of preservation methods by adding natural antimicrobial compound. Antimicrobial compound is used to kill undesired microorganisms in foods or to prevent and inhibit their growth. Thongson *et al.* (2004) stated that one strategy to reduce the number of food borne-illness cases is to apply natural antimicrobial matter during food processing to inactivate or prevent microbial growth.

Essential oil is aromatic substance contained in plants, volatile at room temperature with its plant of origin's typical aroma. In fresh and pure condition, essential oil is colorless, but is oxidized in long storage (Gunawan and Mulyan 2004 *in* Rachmaniar *et al.* 2015). Spice and essential oil offers a promising alternative to replacing chemical preservatives used in food products (Charu Gupta *et al.* 2008).

Aromatic plants and spices are quite important in food, cosmetic and pharmaceutical industries (Sartoratto *et al*. 2004). These industries have shown their great interest in the antimicrobial property of essential oil, since natural additive utilization has become trend to replace synthetic preservatives (Andrade *et al*. 2014). Various essential oils serve to be

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biocide for various organism like bacteria, fungi, virus, protozoa, and insects (Kalemba and Kunicka 2003, Mohammad *et al*. 2009), are evidently environmentally friendly (Chutia *et al.* 2009, Sokovic and Griensven 2006), and may be applied to foods (Madsen and Bertelsen 1995).

Essential oil is volatile, the result of plant's secondary metabolism, obtained from parts of plant such as flowers, leaves, seeds, bark, fruits and roots or rhizomes. It is known that essential oil contains various mixed compounds, including terpene, alcohol, acetone, phenol, acid, aldehyde and ester, commonly used as essence (aroma) additive for foods, cosmetics or as functional component in pharmaceutical products. Various research results and reviews report antimicrobial activity of essential oil derived from spices such as oregano, thyme, sage, rosemary, marjoram, clove, cinnamomum verum, garlic, ginger, turmeric, alpina galanga, black cumin, nutmeg, betel, Etlingera elatior and other spices (Rialita *et al*. 2015). Nutmeg is Indonesia's native spice which has important economic significance is *Myristica fragrans* Houtt (Saputro *et al.* 2016). Nutmeg oil is one essential oil with widely known and exposed potentials.

The widely known nutmeg oil is the essential oil produced from the extraction of nutmeg seed and mace, with useful secondary metabolite for treatment of various infectious diseases (Savena and Patil 2012). Besides seed and mace, the essential oil is also contained in its fruit for about 1.1%, in which 100 grams of the flesh of nutmeg fruit contains water about 10 g, protein 7 g, fat 33 g, essential oil with the main component of monoterpene hydrocarbon (61 - 88% such as α-pinene, β-pinene), monoterpene acid (5 - 15 %), aromatic ether (2 - 18% such as myristicin, safrole) (Nurdjanah 2007). Sipahelut (2012), stated that the advantage of oil derived from the flesh of nutmeg fruit over oil derived from nutmeg seed and mace is that it contains more "oxygenated compounds" (particularly linalool, terpinene-4-ol, and α-terpineol). Oxygenated compound is the main cause of aromatic property in essential oil.

The antimicrobial activity of each type of essential oil is influenced by the type and amount of active components it contains, commonly depending on variety or cultivar, climate and soil where it grows or place of origin, fresh or dry rhizome, as well as extraction method and type of solvent used (Burt 2004).

This research aims at determining the antibacterial and antifungal activity of essential oil derived from the flesh of nutmeg fruit and examining the essential oil's inhibiting activity of the growth of pathogenic bacteria, food-destroying bacteria and food-destroying fungi.

RESEARCH METHOD

Research Materials and Instruments

This research employs completely ripe (about 6 - 7 months from since flowering) flesh of nutmeg fruit (*Myristica fragrans* Houtt) from Maluku as its material and Triptycase Soy Broth (TSB) and Potato Dextrosa Agar (PDA) as its microbial growing media. The other chemical materials and instruments consist of ethanol, paper disc, sterile Aquabides. The test microbial cultures are pure bacterial isolate of *Staphylococcus aureus, Escherichia coli*, and pure fungal culture of *Aspergillus sp, Fusarium moniliforme*.

The instruments in use consist of a set of water-steam distillation apparatus, test tube, Erlenmeyer flask, petri dish, beaker glass, propipet 1 ml, propipet 5 ml, pipet 1 ml, pipet 5 ml, pipet 10 ml, tweezers, incubator, autoclave, UVS cabinet, vortex, calipers.

Extraction of Essential Oil from the Flesh of Nutmeg Fruit

The flesh of nutmeg is chopped and air-dried for one day and put into boiler for extraction through a water-steam distillation process (at 96 ºC for ± 6 hours). The obtained distillate is separated from water phase using anhydrous Na_2SO_4, put in dark bottle and stored at 4°C until usage.

Physiochemical Characterization of Essential Oil Derived from the Flesh of Nutmeg Fruit (ISO 7355:1985)

The essential oil derived from the flesh of nutmeg fruit is analyzed for its physiochemical characteristics, including specific gravity, refractive index, solubility in ethanol, acid value and ester value.

Analysis on the Chemical Composition of Essential Oil Derived from the Flesh of Nutmeg Fruit

The chemical composition of essential oil of the flesh of nutmeg fruit is analyzed using GCMS-QP 2010S Shimadzu. Instrument's operational conditions: column HP-5MS, length 30 meter, ID 0.25 mm, carrier gas helium, ionization EI 70 Ev. Column temperature 70 °C; injection 290 °C, injection mode split, pressure 13.7 kPa, total flow 100 mL/min, column flow 0.50 mL/min, linear velocity 25.9 cm/sec, purge flow 3 mL/min, split ratio 193, ion source 250 °C, interface 300 °C. The constituent compounds are identified using *Library-Wiley 7.LIB.*

Antibacterial Activity Test (Agar Diffusion Method)

24-hour pure bacterial isolate is prepared, in which pure isolate is pipetted for 1 µm and poured into test tube containing 10 ml sterile Triptycase Soy Broth (TSB) solution. The media is incubated at 37ºC for 24 hours. Pure isolate is pipetted for 1 ml and poured onto sterile petri dish. Bacterial growing media is (at 45 ºC) put onto petri dish and shaken so that the bacteria will grow

Fig. 1. Essential oil of nutmeg fruit flesh

Table 1. The physiochemical characteristics of essential oil of nutmeg fruit flesh

Characteristics	Research Results	SNI 06-2388-2006
Specific gravity	0.9054	0.880-0.910
Refractive index	1.486	1.470-1.497
Acid value	4.23	-
Ester value	22.34	-
Solubility in ethanol	Clear and completely dissolved	Clear and completely dissolved

evenly. It is then left until solid. Sterile paper disk is taken, dripped with 100 µl oil of nutmeg fruit flesh with previously added absolute ethanol at ratio of 1:0; 1:1; 1:2 and 1:3, and put onto agar media. The media is incubated at 37 °C for 24 hours. The formed clear zone is measured.

Antifungal Activity Test (Total Plate Count Method)

Potato Dextrosa Agar (PDA) medium is weighted for 10 g and inserted with 225 ml NaCl 0.9%. The PDA medium is sterilized in autoclave for 10 minutes at 115 °C. The PDA medium is put onto petri dish after it is cool, left until solid. 1 dose of each fungus is put into the media on petri dish, and left for a while. Nutmeg fruit flesh oil is dripped pursuant to treatment concentration onto paper disk, put onto the solidified PDA medium. Petri dish is put into plastic, tied, and put into incubator for 2 days at 37 °C. The petri dish is taken out and measured for the inhibitory power of nutmeg fruit flesh-derived oil on fungi using calipers.

RESULT AND DISCUSSION

Characteristics of Nutmeg Fruit Flesh Oil

The essential oil of nutmeg fruit flesh resulted in the research has typical nutmeg oil aroma, thus it generally fulfills the quality standards for nutmeg oil based on SNI 06-2388-2006. According to the visual observation, the produced oil is within the range of colorless to pale yellow, as seen in **Fig. 1**.

Table 2. The Chemical Composition of Essential Oil Derived from the Flesh of Nutmeg Fruit

No	Name	RT (minutes)	Concentration (%)
1	α-thujene	3.225	0.1
2	α-pinene	3.317	14.2
3	Camphene	3.467	0.2
4	β-pinene	3.842	8.2
5	β-myrcene	4.042	3.5
6	α-phellandrene	4.283	2.3
7	β-ocimene	4.383	4.0
8	α-terpinene	4.517	5.5
9	*p*-cimene	4.683	0.5
10	Limonene	4.775	10.9
12	δ-terpinene	5.475	7.3
13	α-terpinolene	6.225	7.5
14	Linalool	6.600	2.2
15	Isoamyl-2-methyl butyrate	6.742	0.5
16	Terpinene-4-ol	8.883	12.4
17	α-terpineol	9.358	13.6
18	Bornyl acetate	12.175	0.0
19	Safrole	12.333	0.6
20	α-copaene	18.725	0.2
21	Myristicin	18.950	13.1

The characteristics of essential oil of nutmeg fruit flesh in this research are given in **Table 1**. The characteristics of produced essential oil of nutmeg fruit flesh fulfill the quality standard parameter pursuant to SNI 06-2388-2006.

The physical characteristics of essential oil such as specific gravity, refractive index, optical rotation and solubility are highly determined by the chemical composition of such oil (Ma'mun 2006). The higher the molecule's weight, it will have higher specific gravity and refractive index, while for acid value, lower acid content in oil will be better. Acid is not desired in essential oil since it is easily changed by air oxidation which causes change in oil aroma. Meanwhile, esters are a valuable component in essential oil since ester compound has desirable aroma. Ester always exists in almost all of essential oils with different concentration.

Chemical Composition of Essential Oil of Nutmeg Fruit Flesh

According to the identification, the nutmeg fruit flesh-derived oil, upon analysis using GC-MS, contains about 21 components (**Table 2**).

The main chemical components existing in the essential oil derived from nutmeg fruit flesh include α-pinene (14.2%), α-terpineol (13.6%), myristicin (13.1%), terpinene-4-ol (12.4%), limonene (10.9%), β-pinene (8.2%), α-terpinolene (7.5%), and δ-terpinene (7.3%).

The components mostly contained in nutmeg fruit flesh-derived oil from the monoterpene hydrocarbon fraction are α-pinene, β-pinene, limonene and δ-terpinene. The components α-pinene and β-pinene are contained more in nutmeg seed and mace-derived oil, while limonene is contained more in nutmeg fruit flesh-derived oil. From the oxygenated monoterpene fraction, the components mostly contained are α-terpineol, terpinene-4-ol and linalool. These components are

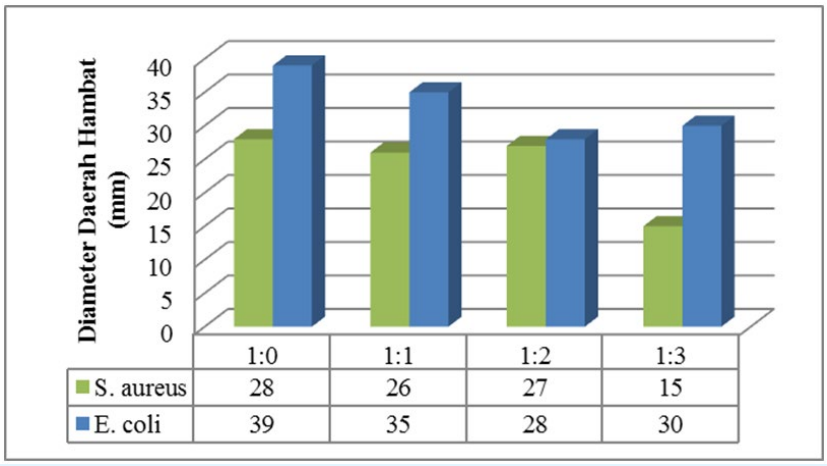

Fig. 2. The Average Antibacterial Inhibitory Power of Nutmeg Fruit Flesh-Derived Oil

contained more in nutmeg fruit flesh-derived oil than in nutmeg seed and mace-derived oil. Meanwhile, myristicin is the component mostly contained from the aromatic ether fraction.

The advantage of essential oil derived from nutmeg fruit flesh is that it contains more oxygenated compounds (particularly terpinene-4-ol, and α-terpineol) than essential oil derived from nutmeg seed and mace (Sipahelut and Telussa 2012). Oxygenated compound is the main cause of aromatic property in essential oil, more resistant and more stable (against oxidation and resinification process).

Antibacterial Activity of Nutmeg Fruit Flesh-Derived Oil

Essential oil actively serving antibacterial function commonly contains hydroxyl (-OH) and carbonyl group. Phenol derivative interacts with bacterial cells through adsorption process involving hydrogen bond. At low content, it forms phenol-protein complexes with weak bond and is immediately decomposed, followed with phenol penetration into cells which cause protein precipitation and denaturation. At high content, phenol causes protein coagulation and membrane cell is lysed (Parwata and Dewi 2008).

The antibacterial activity of essential oil of nutmeg fruit flesh is given in **Fig. 2**. The antibacterial power of essential oil of nutmeg fruit flesh on the growth of bacteria *S. Aureus* and *E. coli* is apparent at all concentrations. This means that the essential oil of nutmeg fruit flesh effectively inhibits bacterial growth.

The antibacterial activity of essential oil is divided into three classes: strong activity (inhibition zone > 8 mm), medium activity (inhibition zone > 6 to < 8 mm), and weak activity (no inhibition zone < 6 mm) (Ela *et al*. 1996 *in* Elgayyar *et al*. 2001). The **Fig. 2**, showed that the use of essential oil derived from nutmeg fruit flesh with any concentration shows strong antibacterial power against all tested bacteria.

The examine result of the diameter of inhibition zone gets smaller with lower concentration of essential oil of nutmeg fruit flesh. Inhibition by antimicrobial compound may be generally caused by: disturbance to components which compose cytoplasm membrane and cell, protein synthesis inhibition, and disturbance to genetic material function (Pelczar 1979 *in* Fadhilla 2010).

Essential oil causes damage to cell membrane. The most important chemical element is the existence of hydrophobic property, accumulated in the cell membrane structure with fat-rich environment, which causes damage to the structure and function of the cell membrane. Essential oil which permeates membrane may coagulate cytoplasm, causing damage to fat and protein (Dorman and Deans 2000). In addition, the essential oil may dissolve phospholipid which composes bacterial cell wall, since the essential oil components have branches of phenol and alcohol groups (Gustafson *et al*. 1998). Any damaged or dissolved phospholipid causes damage to cell membrane, and this damage causes cell leakage. Therefore, any important components such as protein, nucleic acid and nucleotide will escape from bacterial cell, making it unable to do its activity and having its grows inhibited or causing its death (Rupilu and Lamapaha (2008) *in* Sayuti et al. (2014).

The antibacterial ability of matter derived from plant depends on several factors, such as extraction method of essential oil from plant, concentration of essential oil in use, inoculum volume, microbial growth phase, and culture medium in use (Brandi *et al*. 2006, Burt 2004).

Essential oil contains mixed complexes of components, thus it has some antimicrobial characteristics; most of these characteristics are derived from oxygen-containing terpenoid, particularly phenolic terpene, phenylpropanoid and alcohol; other constituent, such as hydrocarbon, commonly showing low activity.

Antifungal Activity of Nutmeg Fruit Flesh-Derived Oil

Conner and Beuchat (1984) *in* Elgayyar (2001) classify essential oil into very active (inhibition zone > 11 mm), relatively active (inhibition zone > 6 to < 11 mm),

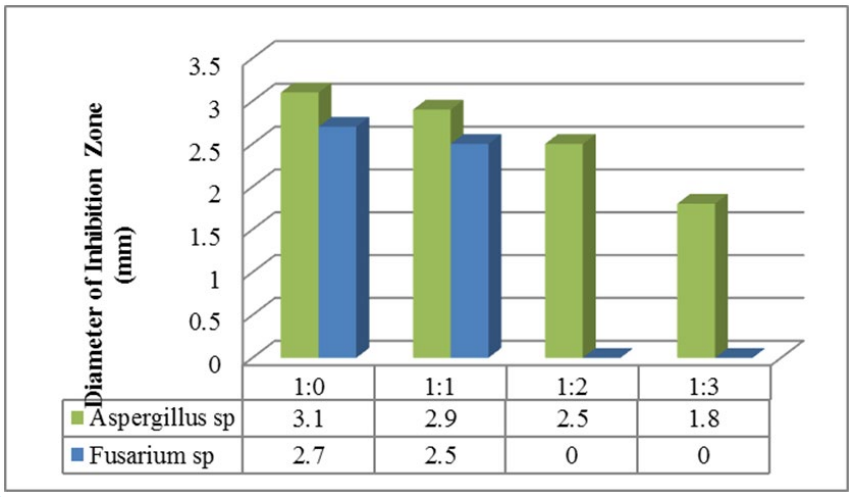

Fig. 3. The Average Antibacterial Inhibitory Power of Nutmeg Fruit Flesh-Derived Oil

or inactive (inhibition zone < 6 mm). The antifungal activity of nutmeg fruit flesh-derived oil is given in **Fig. 3**.

Every concentration of nutmeg fruit flesh-derived oil has average value of growth inhibitory power of fungi *Aspergillus sp*, and *Fusarium sp* in diameter as given in **Fig. 3**, showing that the inhibitory power is weak. Every increment in the concentration of essential oil derived from nutmeg fruit flesh shows increase of inhibitory power, since the higher the concentration of essential oil is given to the medium, the amount of diffused essential oil into fungal cell will increase, causing fungal cell not weak.

The inhibitory power of nutmeg fruit flesh-derived oil on *Fusarium* is classified as weak. For the concentration at ratios of 1:2 and 1:3 on fungus *Fusarium*, there is no inhibitory power value. This shows that the fungus cannot adapt to the given or saturated nutmeg fruit flesh-derived oil, in which oxygen (O_2) is reduced by the mitochondria with damaged cyst because of antifungal compound activity, causing ATP energy produced for cell development and growth process reduced or leading to its death.

According to Griffin (1981), some antifungal compounds may disturb the energy metabolism of mitochondria in the electron transfer and phosphorylation stage. The energy metabolism in mitochondria is inhibited with disturbance of electron transfer. Electron transfer will reduce oxygen and reduce the function of tricarboxcylic acid cycle. Non-existence of phosphorylation stage causes inhibited formation of ATP and ADP.

The antifungal compound contained in the extract of nutmeg fruit flesh-derived oil is allegedly originated from nutmeg fruit flesh-derived oil components which contain compound classified as monoterpene hydrocarbon fraction, including champene, β-mycrene, limonene, α-thujen, α-pinene, β-pinene, β-ocimene, α-terpinene, α-terpinolene with high antioxidant of 73.83 % (Sipahelut and Telussa 2011), thus it has antifungal characteristic.

CONCLUSIONS

According to the research results, we may conclude that:

1. The essential oil derived from nutmeg fruit flesh may inhibit bacterial growth at all concentrations, in which the higher the concentration of nutmeg fruit flesh-derived oil, the higher the antibacterial power it has.
2. The essential oil derived from nutmeg fruit flesh has strong inhibitory power against bacteria *Staphylococcus aureus* and *Escherichia coli.*
3. The essential oil derived from nutmeg fruit flesh has weak inhibitory power against fungi *Aspergillus sp.* and *Fusarium sp.*

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