Antimicrobial Activity and Cytoxicity of Several Sumbawa Traditional Oils (*Minyak Sumbawa*) in Sumbawa Regency, West Nusa Tenggara, Indonesia

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Abstract. Sumbawa oil or *Minyak Sumbawa*, is one of Indonesia's original traditional oils used for more than just external applications and is believed to treat various diseases, from body aches and stomach infections to post-surgery treatment. However, there still needs to be more research that scientifically discusses its medicinal effects. This study evaluates the antimicrobial properties and cytotoxicity of several locally made *Minyak Sumbawa* in Sumbawa Regency, West Nusa Tenggara, Indonesia. *Minyak Sumbawa* from Batu Lanteh (BTL) showed the highest antimicrobial properties against *Salmonella thypi*, *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus* compared to those from Lunyuk (LNK), Pernek (PRN), Taliwang (TLW), Utan (UTN) and from Sumbawa Besar (SBW). In contrast, *Minyak Sumbawa* from Labuan Badas (BDS) has the highest activity against *Staphylococcus epidermidis*. Using the BSLT method, all samples showed low toxicity. The result showed that *Minyak Sumbawa* has various activities against gram-positive and negative bacteria and is safe for consumption overall. Since the oils were made with different recipes, therefore it will affect its chemical constituent. On the other hand, *Minyak Sumbawa* can be modified accordingly for specific health benefits by adjusting and standardizing the recipe to improve the quality of Indonesian traditional medicine.

Keywords: Antimicrobial activity, bioactive compounds, herbal, traditional medicines, traditional medicinal oil.

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1 Introduction

Sumbawa Island is one of the big islands at 8.7381° S, 118.1171° E. One of the original products that are processed by the hands of the Sumbawa people is Sumbawa oil, famously called *Minyak Sumbawa*. *Minyak Sumbawa* is one of Indonesia's original traditional medicines widely used by the community [1]. This oil is made of coconut milk and various herbs cooked until the oil is produced. Making this oil is a culture to the locals, called *Melala*. It is usually held in the month of Muharram, where the experts of *Melala* or locally called *sanro* will gather and demonstrate their skills in making *Minyak Sumbawa* using their recipe and expected to have several medicinal benefits [1, 2]. The locals believe that *Minyak Sumbawa* can cure various diseases. From wounds/skin conditions and stomachache to post-surgery treatments taken externally and orally by the locals [1].

Since *Minyak Sumbawa* is made with herbs, it is expected that bioactive compounds are contributed to its medicinal benefits [1, 3, 4] including being a source of antioxidants, antimicrobial, anti-inflammatory, and anticancer [1, 4]. Unfortunately, few studies have been conducted on the oil and its therapeutic claims. The medicinal uses of this oil are understandable since coconut oil and the herbs used in the recipe have been individually studied and revealed their medicinal purposes. For example, the main ingredient, coconut (*Cocos nucifera* L.) oil is one of the most commonly used oils worldwide for cooking [5], beauty products [6], treatment of skin conditions [7, 8], and dietary supplements to prevent and treat obesity [9, 10], oral health [11], food preservation even for industrial purposes as lubricants [12].

Coconut oils are widely studied and proven beneficial because of several properties, such as antimicrobials [13, 14], anticancer [8, 15], and antioxidants [5, 16, 17]. Hadi *et al*. [2] revealed that the fatty acid content varies from one commercial oil to another (Table 1). Short Chain Fatty Acids (SCFA), commonly found in coconut oils, contribute to their antimicrobial and anticancer properties [18]. Coconut oil contains saturated fatty acids (SFA) (≈ 93 %) and medium-chain fatty acids (MCFA) (≈ 60 %) [5, 19]. MCFA modulates cellular signaling to modify gut microbiota and is accessible in the digestive tract, thus preventing obesity [9]. The supplementation of linoleic acids potentially induces body fat loss and lowers a considerable amount of cholesterol [20]. In addition, lauric acids (C12), generally the highest found in coconut oil, have antimicrobial properties that are effective against gram-positive and negative bacteria and have an immunomodulatory effect [21].

Table 1. Comparisson of fatty acid composition on previously studied coconut oils and *Minyak Sumbawa*.

Fatty acid composition	**Coconut oil (%)** [22]	**Coconut oil (%)** [23]	**Commercial *Minyak Sumbawa (CMS)* (%)** [2]		
			CMS 1	**CMS 2**	**CMS 3**
Caprylic (C8:0)	0.7	5.8 ± 0.4	0.54	0	0
Capric (C10:0)	3.0	4.8 ± 0.3	0.56	3.53	0
Lauric (C12:0)	38.4	49.1 ± 1.6	3.41	27.07	0
Myristic (C14:0)	20.2	21.8 ± 1.1	2.98	16.28	0
Palmitic (C16:0)	13.5	8.4 ± 0.8	9.37	5.79	14.32
Stearic (C18:0)	2.5	2.8 ± 0.2	3.58	8.05	5.45
Elaidic (C18:1 trans9)	Not detected	6.1 ± 0.3	Not detected	Not detected	Not detected
Oleic (C18:1 cis9)	15.5	Not detected	Not detected	Not detected	Not detected
Linoleic (C18:2 cis9,12)	6.1	1.2 ± 0.2	21.07	0	44.7

Table 1 shows that the fatty acid composition of the two hot extracted coconut oils [22, 23] and the three commercial *Minyak Sumbawa* [2] are varied. These fatty acids contribute to their antimicrobial properties [24–27]. Out of the three commercially tested *Minyak Sumbawa*

products, the greatest proportions of fatty acids identified were 27.0 7% lauric acid and 44.07 % linoleic acid. Notably, these values stem from distinct products. The variations in fatty acid compositions among different *Minyak Sumbawa* products appear to be attributed to different methods and herbal formulations [2]. It also shows that during the production, the primary fatty acids typically found in coconut oil is decreasing. These findings underscore the necessity for more in-depth exploration of this traditional oil's health benefits, aiming to establish standardized guidelines of methods, formulations, and potential impacts.

Minyak Sumbawa is used as a wound medicine [1] due to its anti-inflammatory and antimicrobial properties [28]. These properties are desirable in medicine [28]. The herbal composition of *Minyak Sumbawa* has been previously reported by Permatasari [1] and Rahayu and Rustiami [4]. According to Permatasari [1], the ingredients of *Minyak Sumbawa* are coconut milk, ginger (*Zingiber officinale* Rosc.), Moringa roots (*Moringa oleifera* Lam.), chickpeas, roots and leaves of the Saga plant (*Abrus precatorius* L.), as well as seeds and coriander leaves (*Coriandrum sativum* L.). Rahayu and Rustiami [4] added some more ingredients such as cinnamon (*Cinnamomum seylanicum* BI.), white pepper (*Piper nigrum* L.), roots and leaves of Kanekal (*Derris trifoliata* Lour), the bark of Kesambi (*Schleichera oleosa* (Lour) Oken), bark and leaves of Kasokal (*Erioglossum rubiginosum* (Roxb.) Blume), the bark and fruit of the kasene (*Capparis sepiaria* var. Fischeri), and the locally known bark of the Kasela tree. Many of these ingredients have been studied for their antimicrobial, antioxidant and many beneficial properties for health because they are also known and used as food, herbs and medicines in many cultures in Indonesia and many ethnicities in Southeast Asian countries [1, 4, 29, 30].

Traditional medicine is still prevalent in many cultures, and this is demonstrated by the increasing interest in studying its importance and potential in recent years [30, 31]. World Health Organization [31] revealed that the treatment of bones and joints: spine, arthritis, and back pain, was ranked 1st in traditional medicine (87.5 %) in a survey during 2014 to 2019 in several Asian countries. It was also stated that the primary treatment used was massage. The study is in line with the use of *Minyak Sumbawa* as a massage oil in traditional medicine and its also use to treat stomach aches and skin diseases, which also appeared in the survey.

Minyak Sumbawa has not been studied extensively, but each ingredient may reflect its medicinal uses, especially its antimicrobial properties. Ginger has Shogaol and Zingerol bioactive which are found in hot extraction and are effective against *Escherichia coli* (Migula) Castellani & Chalmers, *Staphylococcus aureus* Rosenbach, (Ehrenberg) Cohn, *Aspergillus niger* van Tieghem, *A. terreus* Thom, *Fusarium oxysporum* Schlechtendal and *Rhodotorula* sp. [32–34]. Moringa root has potent antimicrobial activity against *Pseudomonas aeruginosa* (J. Schroter) Migula and *Erwinia carotovora* (Jones) Waldee due to the content of myricetin, quercetin, gentisic acid and biochanin A [35]. Saga beans and roots were found to have very strong activity against *Klebsiella pneumoniae* (Schroter) Trevisan, *S. aureus*, *Streptococcus mitis* Andrewes & Horder emend [36, 37]. Coriander has potent antimicrobial activity against coliform with its many antimicrobial compounds [38, 39]. Kanekal is a mangrove shrubs with high amounts of flavonoids and alkaloids showed activity against *B. subtilis* and *B. coagulans* Hammer [40].

Kesambi stem bark showed potent activity on *S. aureus*, no activity on *E. coli* and low toxicity in all alcohol and water extractions [41]. Bark and leaves of Kasokal showed less activity on the tested bacterial methanol extract but had moderate activity against *B. cereus* Frankland & Frankland, *B. megaterium* de Bary, *Sarcina lutea* (Schroeter) Cohn, *E. coli*, *S. aureus*, *S. paratyphi*, *S. typhi* (Schroter) Warren & Scott, *S. boydii*, *S. dysenteriae* (Shiga) Castellani & Chalmers, *V. mimicus* Davis, *V. parahemolyticus* (Fujino) Sakazaki, Iwanami, & Fukumi, *S. cerevacae* and *C. albicans* (Robin) Berkhout [42], conversely Rana et al. [43] found strong activity against *S. aureus* and *S. typhi* with very low cytotoxicity.

Kasene bark and fruit showed strong activity against *S. aureus*, *Enterococcus faecalis* (Andrewes & Horder) Schleifer & Kilpper-Bolz, *E. coli*, *Proteus vulgaris* Hauser and *C. albicans* and molecular modeling showed that almitic acid, pyrogalol and isopropyl-isothiocyanate are antimicrobial compounds [44] and demonstrated genotoxin potential against *E. coli* [45]. The antimicrobial activity of these plants needs to be analyzed when combined and rarely hot extracted as in the production of *Minyak Sumbawa*. The ingredients used may affect antimicrobial activity thus its potential health benefit, so that it is greatly influenced by the prescribing *sanro*. *Minyak Sumbawa* has been marketed nationally. Unfortunately, its treatment claims will remain claims without further study. This study examined the antimicrobial and cytotoxic properties of several *Minyak Sumbawa* products. This study will also unveil the distribution of *Minyak Sumbawa* within Sumbawa, showcasing the diversity of its production. This research can provide novel prespectives into *Minyak Sumbawa*, offering greater benefits and opportunities for scientific exploration.

2 Materials and methods

2.1 Antimicrobial activity

The antibacterial activity test was conducted by a well diffusion method. *S. thypi*, *L. monocytogenes* (Murray, Webb & Swann) Pirie, *E. coli*, *S. aureus* and *S. epidermidis* (Winslow & Winslow) Evans were collected from National Research and Innovation Agency (BRIN/*Badan Riset dan Inovasi Nasional*) and used as specimens of tested pathogenic bacteria. The bacteria then were cultured on 10 mL of Nutrient Broth media (Oxoid, U.S) and incubated for 24 h at 37 °C 20 µL 0.5 µg μL^{-1} of Ampicillin on paper disc was used as a positive control (MP Biomedicals, USA). 3 mL of bacterial culture test and 20 mL of Nutrient Agar (Oxoid, U.S) were poured into a sterile petri dish. The plates were then allowed to dry and the wells (6 mm in diameter) were made using micro tip. 50 µL of each *Minyak Sumbawa* was put into the well and incubated at 37 °C for 20 h. The diameter of inhibition zone (mm) was measured with criteria of no inhibition zone (0 mm), moderate inhibition zone (5 mm to 10 mm), strong inhibition zone (11 mm to 20 mm), and very strong inhibition zone (> 20 mm) [46]. The inhibition zone indicates that the sample has antibacterial activity. Seven samples of *Minyak Sumbawa* were collected from different villages in Sumbawa Regency, from Batu lanteh (BTL) at 8.5934° S, 117.2393° E; Lunyuk (LNK) at 8.9913° S, 117.2075° E; Pernek (PRN) at 8.5833° S, 117.4263° E, Taliwang (TLW) at 8.7422° S, 116.8499° E; Labuan Badas (BDS) at 8.4735° S, 117.3998° E; Utan (UTN) at 8.4137° S, 117.1313° E; and Sumbawa Besar (SBW) at 8.5040° S, 117.4285° E, as shown in Figure 1.

2.2 Cytotoxic activity and phytochemical screening

Cytotoxicity activity is measured using Brine Shrimp Lethality Test (BSLT) method by counting the mortality rate of shrimp larvae in response to increasing concentration of sample added [47]. Lethal concentration at 50 % mortality rate of the larvae (LC_{50}) indicates cytotoxicity. The experiment was performed in triplets. Whereas the presence of bioactive compounds of *Minyak Sumbawa* in each sample was detected using the colour visualization method using a spectrophotometer for phytochemical screening. Different colours represent different compounds, such as flavonoids, alkaloids, tannins, saponins, quinones, steroids and triterpenoids.

3 Results and discussion

3.1 *Minyak Sumbawa* survey in Sumbawa Island

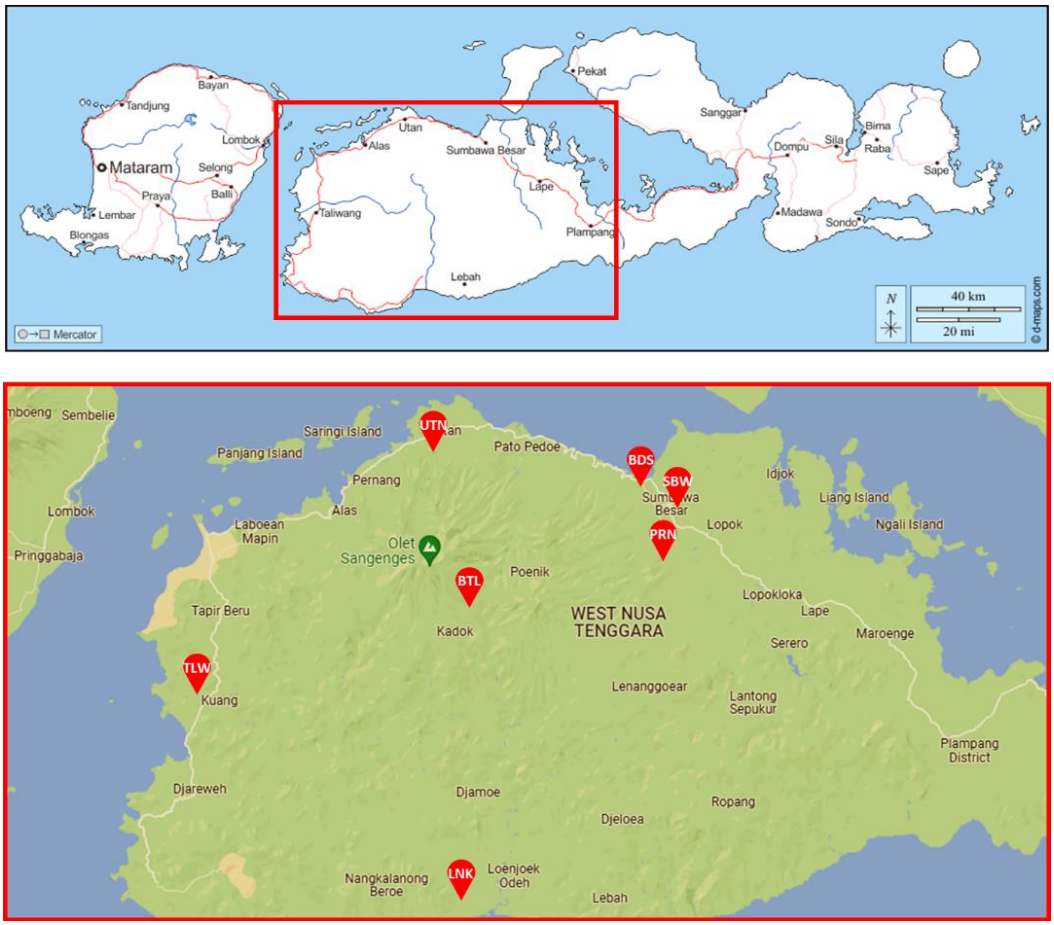

Fig. 1. Distribution of sampling locations of *Minyak Sumbawa* in Sumbawa Regency from Batu lanteh (BTL), Lunyuk (LNK), Pernek (PRN), Taliwang (TLW), Labuan Badas (BDS), Utan (UTN) and Sumbawa Besar (SBW).

Samples were collected from several well-known *Minyak Sumbawa* experts (*sanro*) in Sumbawa Regency, West Nusa Tenggara, Indonesia and conducted interviews. Interviews were conducted to get an overview of the production of Sumbawa traditional oil and the common ingredients used. Many of *sanro* keep some details secret, such as the composition and ratio of herbs to protect product secrets. This is done because many of them are suppliers of commercially sold oils or only produce them through their experience, thus making the oil's chemical constituents different [2].

The herbs were prepared, mostly without precise measurements, according to *sanro*'s wishes. *Sanro* can prepare certain ingredients for specific medicinal purposes. For example, *Minyak Sumbawa* for scars and skin trauma will have different ingredients than those for male stamina and other purposes [48]. Permatasari [1] surveyed three districts in Sumbawa and obtained 59 plants used in *Minyak Sumbawa*. There were five tree barks used in Batu

Dulang village. While ginger, sagaloka, moringa roots, coriander seeds, cinnamon, white pepper were the dominant herbs used [4].

3.2 Antimicrobial activity of *Minyak Sumbawa*

The results showed that each *Minyak Sumbawa* from different productions has different activities. The results of the antimicrobial test of seven samples showed that *Minyak Sumbawa* from Batulanteh (BTL) has the highest overall antimicrobial activity against *S. thypi* (27.00 ± 1.73 mm), *L. monocytogenes* (24.67 ± 1.15 mm), *E. coli* (25.00 ± 2.65 mm) and *S. aureus* (20.67 ± 1.15 mm) and second highest against *S. epidermidis* (18.67 ± 0.58 mm). The lowest antimicrobial activity was SBW against all pathogens as shown in Table 2.

Table 2. Antimicrobial actvity of several *Minyak Sumbawa* towards several pathogenic bacteria.

Sample code	Zone of inhibition (mm)				
	S. thypi	***L. monositogenes***	***E. coli***	***S. aureus***	***S. epidermidis***
BTL	**27.00 ±1.73^{f}**	**24.67 ± 1.15^{d}**	**25.00 ± 2.65^{d}**	**20.67 ± 1.15^{c}**	18.67 ± 0.58^{b}
LNK	17.00 ± 1.00^{cd}	18.33 ± 0.58^{c}	21.67 ± 0.58^{d}	19.33 ± 4.16^{bc}	15.33 ± 1.53^{a}
PRN	14.00 ± 2.00^{ab}	14.33 ± 2.52^{b}	15.67 ± 1.53^{bc}	12.33 ± 0.58^{a}	16.00 ± 1.00^{a}
TLW	15.00 ± 1.73^{bc}	16.00 ± 1.73^{b}	17.33 ± 0.58^{c}	17.67 ± 0.58^{b}	18.67 ± 0.58^{b}
BDS	18.00 ±1.00^{d}	18.33 ± 0.58^{d}	13.00 ± 5.29^{ab}	14.00 ± 1.00^{a}	**19.33 ± 0.58^{b}**
UTN	13.00 ±1.00^{ab}	18.33 ± 0.58^{d}	15.00 ± 1.00^{ab}	14.67 ± 0.58^{a}	16.00 ± 1.73^{a}
SBW	11.67 ± 0.58^{a}	10.00 ± 0.00^{a}	11.33 ± 1.15^{a}	12.00 ± 0.00^{a}	14.67 ± 0.58^{a}
K+	24.00 ± 0.00^{e}	24.00 ± 0.06^{d}	24.00 ± 0.00^{d}	24.00 ± 0.00^{d}	25.00 ± 0.00^{c}

BTL showed the highest antimicrobial activity compared to the positive control, K+ (Ampicillin) against *S. thypi* and *E. coli* suggesting that BTL is potentially more effective against *S. thypi* and *E. coli* infections, as shown in bold. Despite being the lowest antimicrobial activity, SBW showed fairly good antimicrobial activity, within medium to strong activity (10 mm to 15.25 mm) to all bacteria tested (Table 3). *Minyak Sumbawa* is extracted from coconut milk with various locally known medicinal herbs and spices [1]. This simplicia of medicinal herbs and spices usually differ from one *sanro* to another, then be added to fresh coconut milk and heated until the oil forms. By this heat treatment, coconut oil may retain its antimicrobial activity because of its heat-resistant bioactive compounds and fatty acids [16, 23]. Therefore, the added herbs might also strengthen their medicinal properties. Nevertheless, not all bioactive compounds can be extracted this way since some volatile compounds are thermolabile [49].

Heat-resistant bioactive compounds and remaining essential oils can also be extracted in *Minyak Sumbawa*. Tannins, terpenoids and fatty acids are heat resistant and have antimicrobial activity [49]. BTL showed the highest antimicrobial activity against *S. thypi* and *E.coli* compared to the positive control and showed the highest results among the samples against *L. monocytogenes* and *S. aureus*. Plant flavonoids, also present in SBW, have antimicrobial properties against gram-positive and negative bacteria [50]. However, all samples of *Minyak Sumbawa* showed good antimicrobial activity (Table 2).

The fatty acid composition can be tested for future research besides testing the qualitative phytochemicals contributing to antimicrobial activity [2, 22, 23]. Active compounds contained in virgin coconut oil, such as Lauric acid are reported to have antimicrobial

properties against *C. albicans* [13], against gram-positive bacteria, *Staphylococcus* and *Streptococcus* [51], and against gram-negative bacteria such as *E. coli* [14, 52]. A study by Nagase et al. [51] also revealed that VCO with 47 % lauric acid showed higher antimicrobial activity against gram-positive bacteria than synthetic lauric acid. This antibacterial effect is also enhanced by other plant materials that can produce essential oils contributing for healing [24]. The results of this antimicrobial activity require further research that can confirm the antimicrobial content responsible.

3.3 Cytotoxicty of *Minyak Sumbawa*

The BSLT method shows that all samples are low toxicity to shrimp larvae. Using the BSLT method, the cytotoxic properties of all samples are presented in Table 3.

Table 3. Lethal percentage (LC_{50}) of several *Minyak Sumbawa* using BSLT Method.

No.	***Minyak Sumbawa* origin (village)**	Code	**LC_{50} (mg kg^{-1})**	**Toxicity category** [53, 54]
1.	Utan	UTN	489.81	Low
2.	Batu lanteh	BTL	969.43	Low
3.	Pernek	PRN	320.97	Low
4.	Taliwang	TLW	396.02	Low
5.	Lunyuk	LNK	> 1000	Very low
6.	Sumbawa besar	SBW	733.05	Low
7.	Labuhan badas	BDS	982.46	Low

All samples were considered to be of low toxicity as 50 % lethality was achieved at concentrations > 30 mg kg^{-1} and below 1 000 mg kg^{-1} [54], ranging from 320.97 mg kg^{-1} to 982.46 mg kg^{-1}, except for LNY (Table 3). PRN had the highest toxicity among the samples, with 320.97 mg kg^{-1}, and LNY had the lowest toxicity, namely > 1 000 mg kg^{-1}. Terpenoids commonly found in essential oils [54, 55] and coconut oil [56] may exhibit toxicity. Since all samples tested negative for terpenoids, low toxicity might be expected. These results indicate that *Minyak Sumbawa* is tested safe for consumption [53, 55].

4 Conclusion

The results showed that *Minyak Sumbawa* is an effective antimicrobial for clinical pathogen testing, especially in BTL samples. The BTL sample also showed the lowest toxicity effect of all *Minyak Sumbawa* samples, making it safe for consumption. These results indicate that the ingredient composition of *Minyak Sumbawa* can be adapted to provide beneficial medicinal properties and standards for composition and quality. This research has limitations where the ingredient of *Minyak Sumbawa* is a hereditary recipe which is a secret and a hereditary policy from each *sanro*.

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