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Research Article

The antimicrobial study of white turmeric (*Curcuma zedoaria*) extracted using deep euthectic solvent (DES) and ultrasonication

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Keywords

C. zedoaria
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Abstract

This research is prompted by the emergence of various cases of infectious diseases and antibiotic resistance that often occur and the development of the latest research methods in the field of medicinal plants. One of the medicinal plants that is often used is *Curcuma zedoaria* which is generally extracted using conventional solvents. This research aims to find new innovations in the extraction process of medicinal plants. This research is a laboratory experiment carried out in vitro. This research uses the latest solvent, called DES (Deep Eutectic Solvent) with the help of ultrasonication to extract active compounds from *C. zedoaria* as a natural anti-bacterial. DES is known as an environmentally friendly solvent, cheap, easy to make, and is still relatively new in Indonesia. The *C. zedoaria* extraction process uses two types of DES solvent composition assisted by a ultrasonicator. The research results show that: White turmeric extract (*C. zedoaria*) obtained by extraction using DES solvent and ultrasonication. Ultrasonication results using 30%, 70% and 100% DES solvent showed antimicrobial properties against *Escherichia coli* with a lower zone of inhibition compared to the antibiotic ciprofloxacin. Ultrasonication results using 30%, 70% and 100% DES solvent showed antimicrobial properties against *Staphylococcus aureus* with a lower zone of inhibition compared to ciprofloxacin. TLC data shows that the white turmeric extract obtanined contains various antimicrobial substances which allows to inhibit each other to such an extent that their activity is lower than ciprofloxacin.

Introduction

Indonesia is a tropical country which is abundant in various types of traditional medicinal plants that have been used for centuries for generations. Information for the use of traditional medicinal plants is generally passed down orally from generations to generations and the use and research of medicinal plants is currently increasing rapidly along with the emergence of various cases of infectious diseases and frequent antibiotic resistance. One of them is the use of white turmeric as a traditional archipelago plant which is often used by the community empirically (Pacheco-Fernández and Pino, 2019; Subositi and Wahyono, 2019).

White turmeric (*Curcuma zedoaria*) is one of the plants used in traditional medicine. This plant grows well in Indonesia, China, Thailand, Vietnam, and Japan. *C. zedoaria* contains curcumin which has antioxidant, anti-inflammatory, antimicrobial and anticancer properties. In addition, white turmeric has the effect of being a hepatoprotector, neuroprotector, preventing atherosclerosis, and can improve digestive and menstrual disorders. (Teow et al., 2016; Subositi and Wahyono, 2019; Adamczak et al., 2020; Indriani et al., 2020; Kim et al., 2021).

Although research on medicinal plants is increasing, most of the experimental research still uses solvent conventions that are less environmentally friendly and have a high toxic effect. This is what drives the emergence of various studies in search of the latest innovations in creating methods and alternative solvents that are environmentally friendly, have low toxicity, non-toxic, effective, inexpensive, easy to use, and can produce optimal results. One of the newest alternative solvents that has begun to be used is the Deep Eutectic Solvent (DES) which is currently predicted to be an alternative solvent that can replace conventional solvents in

the future. DES is a mixture of an acid and a base or two (or more) solids formed by hydrogen bonds and has a lower melting point which each component has separately. DES also has a low vapor pressure and a high boiling point. This research also focuses on the extraction of phytochemicals and the use of sonication to assist the extraction process. The sonication-assisted DES extraction method is considered having the benefit of producing optimal extraction and stronger antibacterial activity (Bakirtzi et al., 2016; Zainal-Abidin et al., 2017; Kunarto et al., 2019).

Method

Samples, Chemicals, and Materials

This research is a laboratory experiment conducting antibacterial activity tests in vitro. The sample used was white turmeric (*Curcuma zedoaria*) imported from Java, Indonesia. Samples obtained fresh with a sample weight of 5 kg. The white turmeric rhizome is then washed, cut into small pieces, dried for 5 days, and then blended to produce rhizome powder. The rhizome powder was then sieved through a 80 mesh sieve. Afterwards, 700 grams of sample was stored to be processed for the next stage. The chemicals needed include lactic acid solvent, ethylene glycol, glycine, citric acid, aquadest, Nutrient Agar (NA) medium, curcumin standard (Merck). The test bacteria used were *Staphylococcus aureus* and *Escherichia coli*.

The equipment used in this study including beaker glass, measuring cups, basins, scales, gram scales, filter paper, funnels, blenders, hot plate stirrers, handscoens, rotary evaporators (Heidolph), glass bottles, petri dishes, circular needle, circulating water bath, incubator, test tube, autoclave, aluminum foil, sieve, sterile gauze/ cotton swab, label stick, tongs, stir bar, storage vessel, amber glass extractor, freezer, volume pipette, GCMS devices, ultrasonicator (OmniSonic Ruptor 400), FTIr, vacuum pumps (Krisbow vacuum pumps), column chromatography equipment, thin layer chromatography equipment (Supelco TLC Silica gel 60 F_{254}).

Sample Separation

The process of separating samples to see the content of curcuminoid compounds or the number of other antibacterial compounds contained in the sample. The separation process was carried out via column chromatography using silica gel and chloroform:dichloromethane as eluent in a ratio of 2:3. Thin layer chromatography profile of the extract using chloroform:dichloromethane as eluent with a ratio of 32.5:67.5 v/v (Endarini, 2016; Julianto, 2019; Kautsari et al., 2020).

Deep Eutetic Solvent Preparation

Deep eutectic solvents (DES) are solvents consisting of two or three solvent components mixed in the right ratio to reach the eutectic point. In this research, researchers used two types of DES, each of them has different components and solvent ratios. The first DES component (called DES-1) consists of lactic acid and glycine with each molar ratio of 3:1, while the second DES component (called DES 2) consists of a mixture of citric acid and ethylene glycol with each molar ratio of 1:4. The two components of DES and water had been added at a predetermined molar ratio and mixed in a water bath at 90° Celsius. The mixture stirred until it forms a clear liquid (30-90 minutes). Previous research conducted by Bakirtzi et al. (2016) on medicinal plants native to Greece proved that a mixture of lactic acid, glycine and water showed high extraction efficiency (Bakirtzi et al., 2016, Zainal-Abidin et al., 2017; Qin et al., 2020, Rachmaniah et al., 2020; Kurtulbaş et al., 2020).

White turmeric samples were then weighed and mixed based on the optimum time and composition in accordance with optimization studies in research. Extraction step using DES solution from white turmeric (*Curcuma zedoaria*) (Candani et al., 2018; Rachmaniah et al., 2020). After the extraction preparation process was complete, the two DES fractions were collected and sonicated using an ultrasonic machine (Omni Sonic Ruptor 400 Ultrasonic Homogenizer) for 15 minutes.

Antibacterial Test

This study used the disc diffusion method to test the effectiveness of white turmeric antibacterial compounds from DES extract. The agar medium used was Nutrient Agar (NA) media. *E. coli* and *S. aureus* bacteria were then inoculated on agar media using the swap technique. Curcuma zedoaria extract at concentration of 30%, 70%, 100% was tested for its ability to inhibit the growth of each bacteria. The larger the diameter of the inhibition zone, the stronger the antibacterial effect is going to be. There are five groups in this experiment. The first group is a negative control containing aquadest; the second group which is a positive control and contains

ciprofloxacin. The third, fourth, and fifth groups containing Curcuma zedoaria extract with respective concentrations of 30%, 70%, and 100%. The diameter of the inhibition zone was measured, then compared with the inhibition zone standard to determine the level of antimicrobial strength according to Greenwood (Indriani et al., 2020; Tjiptoningsih, 2021).

Results and Discussion

The Results of Sonication and Extraction of Curcuma zedoaria Using DES Solvent

The white turmeric is thinly sliced and then left for 5 days at room temperature to be completely dried. During drying processing, avoid exposure to sunlight so that the antimicrobial substances contained in it a will not be damaged, and the results can be seen in Fig.-1a. The dried turmeric were ground to 80 mesh size using a Retsch Testsieve sieve. From 7 kg of white turmeric, 600 g of rhizome flour is obtained as shown in Fig.-1b.

DES1 has been obtained by mixing lactic acid with glycine and DES2 by mixing citric acid with ethylene glycol. The mixture had been diluted with added the water. The molar ratio of the DES mixture containing lactic acid, glycine and water is 3:1:3 respectively. The molar ratio of the DES mixture containing citric acid and ethylene glycol is 1:4. The water had added at a ratio of 20% v/v of the mixture. The two components of DES and water had been added at a predetermined molar ratio and mixed in a water bath at 90° Celsius as shown in Fig.-1c. White turmeric extract was prepared by dissolving turmeric powder into a DES plate then sonicated using ultrasonic machine (Omni Sonic Ruptor 400 Ultrasonic Homogenizer) for 15 minutes as shown in Fig.-1d.

a b c d

Fig.-1. Sample preparation with hot plate stirrer and ultrasonication exctract. a: The turmeric sample has been cut into small pieces and is in the process of being dried; b: Turmeric sample after blending and sifting; c: Preparation of DES samples according to composition with a hot plate stirrer; d: Ultrasonication uses an ultrasonic device.

The Result of Antibacterial Test from white Turmeric DES Solvens and Ultrasonication Extract

To determine the inhibition of white turmeric rhizome extract (*C. zedoaria*) with concentrations of 30%, 70%, and 100% against *S. aureus* (gram-positive bacteria) and *E. coli* (gram-negative bacteria) (Table 1). Inhibitory power was indicated by the growth of *S. aureus* and *E. coli* bacteria around the disc which was in the form of a bright zone. Furthermore, the bright zone will be measured with a digital vernier caliper in millimeter units. Based on the table of standard categories of antibacterial power proposed by David and Stout, the level of antibacterial inhibition by substances contained in white turmeric extract can be seen in Table 2.

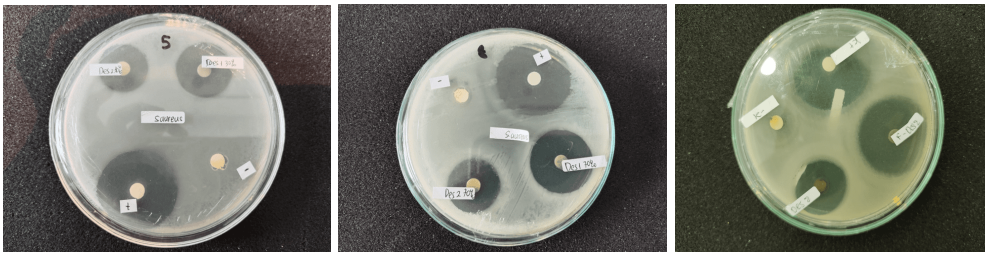
Table 1. Diameter of inhibition zone by DES extract of *C. zedoaria*

Group	Diameter of Inhibition Zone (mm)			
	Ciprofloxacin	Concentration (%)		
		30%	70%	100%
CA-EG on *S. aureus*	27.0	16.50	17.50	19.5
CA-EG on *E. coli*	31.50	17.50	20.50	21.50
LA-G on *S. aureus*	26.50	17.50	20.50	22.50
LA-G on *E. coli*	31.0	17.50	18.50	22.50

Table 2. Level category of antimicrobe on turmeric extract based on their inhibition zone diameter

Group	The category of inhibition zone diameter (according to David and Stout)			
	Ciprofloxacin	Concentration (%)		
		30%	70%	100%
CA-EG on *S. aureus*	Very Severe	Severe	Severe	Severe
CA-EG on *E. coli*	Very Severe	Severe	Very Severe	Very Severe
LA-G on *S. aureus*	Very Severe	Severe	Severe	Very Severe
LA-G on *E. coli*	Very Severe	Severe	Very Severe	Very Severe

The Table 1 and Table 2, shows that of the 4 test treatments there were 12 samples of *C. zedoaria* 30%, 70%, and 100% and 1 sample of ciprofloxacin which had a bacterial growth inhibitory response. Based on the explanation above, it can be concluded that the extracts of *C. zedoaria* and ciprofloxacin both have inhibitory power against the growth of *S. aureus* and *E. coli* with severe and very severe level category. The inhibition zone of DES 1 (CA-EG) and DES 2 (LA-G) extracts against Staphylococcus aureus at various concentrations can be seen in Fig.-2.

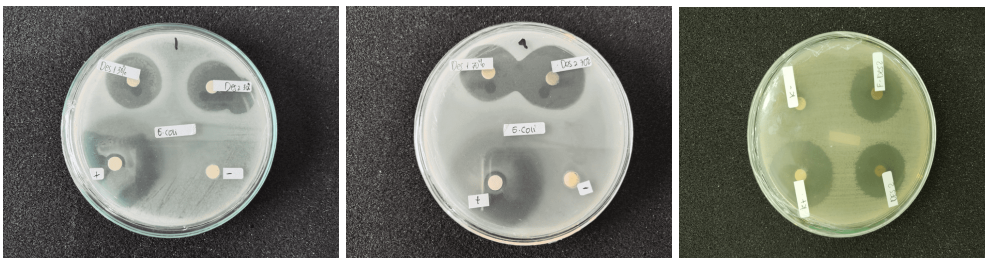


a b c

Fig.-2. The inhibition zone extracts DES 1 (CA-EG) and DES 2 (LA-G) respectively with a concentration of 30% (a), 70% (b) and 100% (c) containing positive control (+), specifically Ciprofloxacin and negative control (-) specifically aquadest against *S. aureus*.

Generaly, the inhibition of antimicrobial substances present in white turmeric extract was still lower than ciprofloxacin as a positive control. This is understandable because the antimicrobial substances are still mixed which allows mutual inhibition to occur. Based on the results in Table 2, it can be seen that S aureus has the inhibition of DES 1 extract (CA-EG) with concentrations of 30%, 70% and 100% which was in severe category and ciprofloxacin was in the very severe category. Furthermore, for and DES 2 (LA-G) with a concentration of 30%, 70% is in the severe category while 100% is very severe like as ciprofloxacin. The inhibition zone of DES 1 (CA-EG) and DES 2 (LA-G) extracts against *E. coli* at various concentrations can be seen in Fig-3.

Table 2 also shown that *E. coli* has the inhibition of both DES 1 extract (CA-EG) and Des2 (LA-G) with concentrations of 30% in severe category but on 70% and 100% shown in very severe like as ciprofloxacin. From the results obtained it can also be seen that the antimicrobial activity of white turmeric extract is stronger against *E. coli* than *S. aureus*.



a b c

Fig.-3. The inhibition zone extracts DES 1 (CA-EG) and DES 2 (LA-G) respectively with a concentration of 30% (c), 70% (d) and 100 % (e) containing positive control (+), specifically ciprofloxacin and negative control (-) specifically aquadest against *E. coli*

Previous research conducted by Busman et al. (2019) on the antibacterial activity of *C. zedoaria* rhizomes against *S. aureus* bacteria using DMSO solvent showed that *C. zedoaria* rhizomes had the strongest antibacterial activity against *S. aureus* bacteria at concentrations of 60% (15.85mm) and 80% (22.20 mm) with respective categories of strong and very strong inhibitory power. Meanwhile, in the research conducted by the author, it was found that the strongest inhibitory power of DES 1 extract was at a concentration of 70% (20.5mm) and 100% (22.5mm) with a very strong inhibitory power category. In the DES 2 extract, the strongest inhibitory power was also found at concentrations of 70% (17.5mm) and 100% (19.5mm). This is also supported by Gharge et al. (2021) who shows that white turmeric extract has inhibitory power against bacteria.

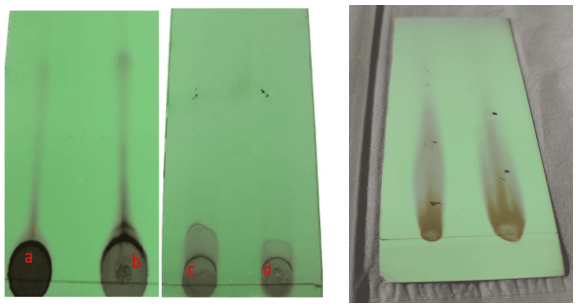
Research conducted by Das and Rahman (2012) showed that methanol extract of *C. zedoaria* rhizomes had an inhibitory power against *S. aureus* of 13 mm and an inhibitory power against *Eshericia coli* of 11 mm (Silalahi, 2020). *C. zedoaria* rhizome pet ether extract also has an inhibitory power against *S. aureus* of 13 mm and an inhibitory power against *E. coli* of 11 mm. Likewise, research conducted by Pangestika et al. (2020) found that *C.*

zedoaria methanol extract had an inhibitory power of 16 mm against *S. aureus* of 16 mm and against *E. coli* of 11 mm. These results indicate an increase in antibacterial inhibitory power against gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*E. coli*) using DES extract and assisted by ultrasonication.

The TLC data of Curcuma zedoaria DES Extract

Separation using the column chromatography method is to determine the composition of the compounds contained in the sample. The eluent used for column chromatography was a mixture of chloroform and dichlormethane with a ratio of 2:3 to modify polarity. The column was filled with 50 grams of silica, then filled with 5 ml of turmeric sample and then elution was carried out continuously until 4 fractions were obtained from DES-1 and 2 fractions from DES-2.

Furthermore, TLC was carried out to see the diversity of chemical substances in white turmeric extract resulting from column chromatography. Approximately 10 µL of the extract was spotted on the TLC plate which had been marked in the form of a line with a length of 5 mm. The TLC plate was inserted into the chamber which had previously been saturated with the mobile phase and then each eluent was allowed to move from the line that had been made. The results of identification through the TLC plate found that in DES 1 there were 2-3 stains and in DES 2 there were 3 spots, which indicated that there were still around 2-3 metabolites in the sample by thin layer chromatography as shown in Fig.-4.



a b

Fig.-4. The TLC data of DES1 and DES-2 *C. zedoaria* extract. a: TLC data of DES-1; and b: TLC data of DES2

The results of separation using TLC showed that there were several metabolite compounds contained in the sample which made it possible for there to be antibacterial compounds contained in the sample. Research conducted by Dosoky and Setzer (2018) also shows that there are quite a lot of metabolite compounds contained in *C. zedoaria*.

Conclusion

The white turmeric extract (*C. zedoaria*) has been obtained by extraction using DES solvent and ultrasonication. Ultrasonication results using DES solvent 30%, 70% and 100% respectively showed antibacterial properties for *E. coli* with a lower inhibition zone compare than Ciprofloxacin. Ultrasonication results using DES solvent 30%, 70% and 100% respectively showed antbacterial properties for *S. aureus* with lower inhibition zones compared than ciprofloxacin. From the TLC data, it was shown that the white turmeric extract obtained contains various antibacterial substances that allowed to inhibit each other to such an extent that their activity was lower than that of ciprofloxacin.

Conflict of Interests

The author (s) declares that there is no conflict of interest in this research and manuscript.

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References

Adamczak, A., Ożarowski, M., & Karpiński, T. M. (2020). Curcumin, a natural antimicrobial agent with strain-specific activity. *Pharmaceuticals*, 13(7), 153. https://doi.org/10.3390/ph13070153

Bakirtzi, C., Triantafyllidou, K., & Makris, D. P. (2016). Novel lactic acid-based natural deep eutectic solvents: Efficiency in the ultrasound-assisted extraction of antioxidant polyphenols from common native Greek medicinal plants. *Journal of Applied Research on Medicinal and Aromatic Plants*, 3(3), 120–127. https://doi.org/10.1016/j.jarmap.2016.03.003

Busman, B., Edrizal, E., & Wirahmi, S. D. (2019). Daya hambat ekstrak rimpang temu putih (Curcuma zedoaria) terhadap Streptococcus mutans dan Staphylococcus aureus. *Menara Ilmu*, 13(6), 19-28.

Candani, D., Ulfah, M., Noviana, W., & Zainul, R. (2018). *A review pemanfaatan teknologi sonikasi*. FMIPA Universitas Negeri Padang, Padang.

Das, K., & Rahman, M. A. (2012). Analgesic and antimicrobial activities of Curcuma zedoaria. *Int J Pharm Sci*, 4(Suppl 5), 322-328.

Dosoky, N., & Setzer, W. (2018). Chemical composition and biological activities of essential oils of curcuma species. *Nutrients*, 10(9), 1196. https://doi.org/10.3390/nu10091196

Endarini, L.H. (2016). *Farmakognisi dan fitokimia, pusdik SDM kesehatan*. Jakarta: Kementerian Kesehatan Republik Indonesia.

Gharge, S., Hiremath, S. I., Kagawad, P., Jivaje, K., Palled, M. S., & Suryawanshi, S. S. (2021). Curcuma zedoaria Rosc (Zingiberaceae): a review on its chemical, pharmacological and biological activities. *Future Journal of Pharmaceutical Sciences*, 7(1). https://doi.org/10.1186/s43094-021-00316-1

Indriani, V., Chiuman, L., Wijaya, L. L., Lister, G., & Grandis, L. (2020). Antibacterial effect of Curcuma zedoaria Extract on Bacillus cereus and Staphylococcus epidermidis. *Althea Medical Journal*, 7(1), 6–10. https://doi.org/10.15850/amj.v7n1.1886

Julianto, T. S. (2019). Fitokimia tinjauan metabolit sekunder dan skrining fitokimia. *Journal of Chemical Information and Modeling*, 53, 1-116.

Kautsari, S. N., Purwakusumah, E. D., & Nurcholis, W. (2021). Profil kromatografi lapis tipis ekstrak kunyit (Curcuma longa Linn) segar dan simplisia dengan variasi metode ekstraksi. *Media Farmasi*, 16(1), 65. https://doi.org/10.32382/mf.v16i1.1403

Kunarto, B., Sutardi, S., Supriyanto, S., & Anwar, C. (2019). Optimasi ekstraksi berbantu gelombang ultrasonik pada biji melinjo kerikil (Gnetum gnemon L., 'Kerikil') menggunakan response surface methodology. *Jurnal Aplikasi Teknologi Pangan*, 8(3), 104. https://doi.org/10.17728/jatp.5122

Kurtulbaş, E., Pekel, A. G., Bilgin, M., Makris, D. P., & Şahin, S. (2020). Citric acid-based deep eutectic solvent for the anthocyanin recovery from Hibiscus sabdariffa through microwave-assisted extraction. *Biomass Conversion and Biorefinery*, 12(2), 351–360. https://doi.org/10.1007/s13399-020-00606-3

Kim, K. M., Lee, J. Y., Jeon, B. H., Quan, K. T., Na, M., Nam, K.-W., & Chae, S. (2021). Extract of Curcuma zedoaria R. prevents atherosclerosis in apolipoprotein E-deficient mice. *Nutrition Research and Practice*, 15(3), 319. https://doi.org/10.4162/nrp.2021.15.3.319

Pacheco-Fernández, I., & Pino, V. (2019). Green solvents in analytical chemistry. *Current Opinion in Green and Sustainable Chemistry*, 18, 42–50. https://doi.org/10.1016/j.cogsc.2018.12.010

Pangestika, I. I. J., Widayati, A., Hartati, E. L. K. N., & Yugatama, A. (2020). Aktivitas antibakteri pada rimpang famili Zingiberaceae terhadap Staphylococcus aureus dan Escherichia coli. *Proceedings National Conference PKM Center*, 1, 144-149.

Qin, H., Hu, X., Wang, J., Cheng, H., Chen, L., & Qi, Z. (2020). Overview of acidic deep eutectic solvents on synthesis, properties and applications. *Green Energy & Environment*, 5(1), 8–21. https://doi.org/10.1016/j.gee.2019.03.002

Rachmaniah, O., Istiqomah, I., Fakhrinanda, F., & Rachimoellah, M. (2020). Central composite design based statistical modelling of curcuminoids extraction from Curcuma zedoaria using choline chloride based Natural Deep Eutectic Solvent (NADES). *IOP Conference Series: Materials Science and Engineering*, 823(1), 012011. https://doi.org/10.1088/1757-899x/823/1/012011

Teow, S.-Y., Liew, K., Ali, S. A., Khoo, A. S.-B., & Peh, S.-C. (2016). Antibacterial action of curcumin against Staphylococcus aureus: A brief review. *Journal of Tropical Medicine*, 2016, 1–10. https://doi.org/10.1155/2016/2853045

Silalahi, M. (2020). Curcuma zedoaria (Christm.) roscoe (benefits and bioactivity). *Eureka Herba Indonesia*, 1(2), 44–52. https://doi.org/10.37275/ehi.v1i2.10

Subositi, D., & Wahyono, S. (2019). Study of the genus Curcuma in Indonesia used as traditional herbal medicines. *Biodiversitas Journal of Biological Diversity*, 20(5). https://doi.org/10.13057/biodiv/d200527

Tjiptoningsih, U. G. (2021). Uji daya hambat air perasan buah lemon (Citrus limon (L.) BURM. F.) terhadap pertumbuhan bakteri aggregatibacter actinomycetemcomitans. *Jurnal Ilmiah dan Teknologi Kedokteran Gigi*, 16(2), 86–96. https://doi.org/10.32509/jitekgi.v16i2.1100

Zainal-Abidin, M. H., Hayyan, M., Hayyan, A., & Jayakumar, N. S. (2017). New horizons in the extraction of bioactive compounds using deep eutectic solvents: A review. *Analytica Chimica Acta*, 979, 1–23. https://doi.org/10.1016/j.aca.2017.05.012