Antibacterial and Characterization of Secondary Metabolite Compound from Ethyl Acetate and Ethanol Fraction of Leaves *Moringa oliefera* L

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Keywords: Fractionation, *Moringa oleifera* leaves, Secondary metabolite compounds, Antibacterial.

Abstract: The microorganisms that cause infections can mutate due to excessive antibiotic exposure. One of the new drug search strategies is through the exploration of active ingredients derived from plants that have been used empirically by the community. *Moringa oleifera* L leaf is a plant that has been used and has been shown to have antibacterial, antifungal, analgesic, and antihypertensive activities. *Moringa oleifera* L leaves contain secondary metabolites such as alkaloids, tannins, saponins, flavonoids, and phenols. The purpose of this study was to obtain active ingredients from the leaves of *Moringa oleifera* L which will be used as a Standardized Herbal Medicine product in the treatment of infectious cases. In order to get the active ingredient as an antibacterial, multilevel extraction is carried out using different polarity solvents, so that a fraction containing nonpolar, semipolar and polar compounds will be obtained. Antimicrobial potential will be tested on each fraction using the disk diffusion method. The results of identification of the compounds in the ethyl acetate fraction show the class of compounds Flavonoids, Terpenoids, Polyphenols and Anthraquinone while in the ethanol fraction *Moringa oleifera* L. leaves show the compounds of the compounds Alkaloids, Flavonoids, Terpenoids, Polyphenols, and Saponin. Antimicrobial activity is shown in the ethyl acetate and ethanol fraction in both Staphylococcus aureus and Escherichia coli.

1 INTRODUCTION

Infection is a pathological condition caused by microorganisms such as bacteria, viruses, fungi, and protozoa, and can occur in the community or in hospitals. Patients who are being treated at the hospital, have a greater risk of contracting the infection than outside the hospital. This can occur as a result of interactions between patients, environments, and microbes showed that 10 rooms out of 16 inpatient rooms in the "X" hospital in Semarang City had airborne germ exceeding the total threshold of germs in the inpatient room (Wikansari, 2012). Infections that occur in hospitals and attack patients who are in the process of treatment are known as nosocomial infections. The prevalence of nosocomial infections in Indonesia is 7.1% (Wikansari, 2012). Various attempts have been made by the hospital in dealing with nosocomial infections namely by washing hands before and after contact with patients; use personal protective equipment such as gloves, masks, and other personal protective equipment; decontaminate equipment after use in service; sharp tool management; medical and non-medical waste management.

Handling in cases of infection is antibiotic therapy. But now some antibiotics are no longer able to deal with cases of infection because they are caused by antibiotic resistance. This antibiotic resistance occurs because of the use of antibiotics freely, so that microorganisms become more resistant to antibiotics. The resistance of microorganisms to antibiotics occurs because they are too often exposed to antibiotics so that microorganisms undergo mutations to form a biofilms layer so that the cell walls are thicker and cannot be penetrated by antibiotics. This mechanism is a self-defense mechanism for microorganisms to be able to survive (Brooks *et al.*, 2013). The highest resistance found in antibiotics penicillin and cephalosporin first generation (Yacob *et al.*, 2011).

This antibiotic resistance if left untreated can cause cases of infection to become uncontrollable.

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The increase in incidence will be very rapid because infection is a contagious disease. Efforts made to prevent antibiotic resistance are through the establishment of antibiotic use policies only in diseases which according to laboratory data have indeed been proven to be due to microorganisms. Another effort undertaken is to explore new medicines with plant sources that have been proven empirically efficacious as antimicrobials. Secondary metabolite compounds that have been shown to have antimicrobial properties are a group of phenolic compounds such as simple phenols, phenolic acids, quinones, flavones, flavonoids, tannins, coumarin; terpenoid compounds and essential oils; and alkaloids (Luqman *et al.*, 2012; Akinyeye, Solanke and Adebiyi, 2014; Gyawali and Ibrahim, 2014; Minaiyan *et al.*, 2014).

One of the plants that has been empirically proven as an antibacterial is *Moringa oleifera* L. This plant contains alkaloids, tannins, saponins, flavonoids, and phenols (Oluduro, 2012). *Moringa oleifera* leaf extract has antimicrobial activity against bacteria Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, and Streptococcus pneumonia (Kalpana, Moorthi and Kumara, 2013). The study was conducted using agar diffusion methods and extracts used 200-800mg / ml. The solvent used is chloroform, petrolatum ether, ethanol, and water. From these studies, the results showed that all extracts showed antimicrobial activity. At a concentration of 800mg the average inhibition zone showed Klebsiella pneumonia 9.3 ± 0.46, Escherichia coli 11.0 ± 0.00, Staphylococcus aureus 13.0 ± 0.00, and Streptococcus pneumonia 7.0 ± 0.81. The ethanol extract showed that the maximum inhibition zone was S. aureus and the smallest inhibitory zone water extract was in Streptococcus pneumonia. For this study, the positive control used was tetracycline 10 mcg.

Based on the results of previous studies show that the leaves of *Moringa oleifera* L. have the potential as raw materials for standardized herbal medicines as antimicrobials. Raw materials for standardized herbal medicines can be in the form of extracts or fractions resulting from the separation of plant secondary metabolite compounds (Sarker, Latif and Gray, 2006). Based on research conducted by Rofida, et al (2017) that the fractionation process of Garcinia mangostan Linn extract can increase its cytotoxicity effect on T47D cell culture. This shows that there is an accumulation of active compounds in the extraction fraction.

In order to obtain active ingredients from *Moringa oleifera* L. leaves, multilevel extraction will be carried out using multilevel extraction techniques using solvents that have different polarities so that non-polar compounds, semipolar compounds, and polar compounds will be obtained. Multilevel extraction using different polarity solvents will separate secondary metabolites based on their solubility. Groups of base form alkaloids, free flavonoids, and free terpenoids will be more easily extracted with nonpolar to semipolar solvents. Whereas the alkaloids in the form of salts, flavonoids glycosides, terpenoid glycosides, and polyphenols are more easily extracted in polar solvents (Sarker et al., 2006). The fraction of the results of the separation will be tested for antimicrobial potential, especially in gram-positive bacteria (Staphylococcus aureus) and gram-negative (Escherichia Coli) in vitro by disc diffusion method. Furthermore, the active ingredient of *Moringa oleifera* L. leaves which has potential as an antimicrobial will be characterized by secondary metabolite compounds by the TLC method.

2 METHODS

Material

Moringa oleifera leaves L., Staphylococcus aureus Escherichia Coli, Silica Gel TLC Plate GF 254, Ethyl acetate, Ethanol, Mueller Hinton Agar medium.

Fractionation

Moringa oleifera L. leaf powder was extracted stratified with hexane, ethyl acetate, and ethanol as solvent respectively with maceration technique. The ingredients are soaked in hexane solvent for 24 hours, then filtered and separated the filtrate. The residue is given the same treatment. This treatment is repeated until the filtrate does not show stains on the silica plate. The filtrate obtained was concentrated using a rotary evaporator at 50°C until a thick extract was obtained. Each viscous fraction obtained was dried in an oven at 40°C. Each thick fraction ready for use is stored in a refrigerator at 5-8°C.

Preparation Sample

The test solution to be used was made by weighing each fraction of *Moringa oleifera* L. as much as 50 mg, 25 mg, 12.5 mg dissolved in 0.1 ml of 1% DMSO then added with sterile aquadest to 1 ml to obtain the concentration of the test solution in the amount of 50 ml / mL; 25 ml / mL; 12.5 mg / mL

Antibacterial Activity by Disk Diffusion Method

The test was carried out by filling sterilized Petri dishes with nutrient media to as much as 20 mL and

waiting for it to harden. Then the bacteria is applied, evenly with the streak plate method. The media used for bacterial culture is sterile aquadest that has been standardized with McFarland's standard (106 CFU / ml) (McFarland, 1907). The hexane, ethyl acetate and ethanol fractions of M. oleifera leaves were weighed 50mg each dissolved in 1 ml of solvent (according to the solvent's flavor) and then bottled on the TLC plate as much as 5 µl. The hexane fraction was eluted with n-hexane: ethyl acetate: formic acid (6.5: 3.5: 3 drops) mobile phase, the ethyl acetate fraction used the n-hexane: ethyl acetate (4: 6) mobile phase system and ethanol fraction uses a mobile phase system of n-hexane: ethyl acetate: methanol (0.5: 4: 0.5) plus 1 drop of formic acid. The spot stains that are covered are cut and sterilized for 30 minutes using UV light in LAF (Laminar Air Flow). The TLC plate is then planted in a petri dish that contains bacterial cultures. Petri dishes are incubated for 24 hours at 37oC. Then the inhibition zone is formed. Antibacterial testing was replicated three times. As a positive control, erythromycin 15 µg / disk was used. As a negative control, TLC plates were used which were incubated without any test material spills.

Characterization of Secondary Metabolite Compounds with Thin Layer Chromatography Methods

Each extract produced was carried out by thin-layer chromatography test using silica gel F254 stationary phase and various eluent mobile phases. The TLC profile was observed with UV lamps 254 and 365. To find out the class of compounds, the TLC results were derivatized with dragendorf solvent, anisaldehyde-sulfuric acid, FeCl3, KOH, and 10% H2SO4.

3 RESULTS AND DISCUSSION

From the concentration process, it was obtained that the ethyl acetate fraction of M. oleifera leaves was as thick as 7.83 g yield of the fraction produced was 3.132%. The yield of ethanol fraction was 42.65 g, the yield of the fraction extracted from M. oleifera leaves was 17.06%. Antibacterial activity of M. oleifera L. leaves
fractionation against Staphylococcus aureus and Escherichia Coli bacteria can be seen in Table 1. The identification test carried out on M. oleifera leaf ethyl acetate fraction using thin-layer chromatography (TLC) method showed that there were no alkaloid compounds. The results of the TLC test can be seen in Figure 1. Based on table 1, the ethl acetate fraction and ethanol fraction showed that the compound with Rf 0.9 showed the highest antibacterial activity against Staphylococcus aureus Escherichia Coli. Gram-negative bacteria have a way to protect their cell membranes from penetrating antibacterial agents, because they have a unique outer membrane, relatively thinner peptidoglycan walls, and periplasmic space between the cell wall and membrane. This outer membrane structure contains Lipopolysaccharides (LPS) or endotoxins, a complex structure consisting of Lipid A, short chains of sugar and long chains of carbohydrates called O-antigens. O antigens and polysaccharides contained in bacterial outer membranes play a role in preventing the penetration of hydrophobic compounds , such as anthraquinone compounds, into the cell membrane, while the penetration of hydrophilic compounds, such as phenol and tannin compounds, into the cell membrane is prevented by the lipid properties they have (Brooks *et al.*, 2013).

The results of identification by TLC method against the ethyl acetate fraction of M.oleifera leaf showed that there was a class of terpenoids compounds (Figure 1), flavonoids compounds (Figure 2), polyphenol compounds (Figure 3), anthraquinone compounds (Figure 4). The results of identification of compounds by TLC technique on spot color observation both visually and irradiated by UV 254 nm and 365 nm, obtained Rf value presented in Table 2. In previous studies it was found that the ethyl acetate fraction of M. oleifera leaves contained chemical compounds such as alkaloids, flavonoids, saponins, tannins, terpenoids (Moyo, Masika and Muchenje, 2012; Kalpana, Moorthi and Kumara, 2013; Abdallah, 2016).

The results of identification by TLC method against the ethanol fraction of M.oleifera leaf showed that there was a class of polyphenol compounds (Figure 5), alkaloids compounds (Figure 6), flavonoids compounds (Figure 7), saponin compounds (Figure 8), terpenoids compounds (Figure 9). The results of identification of compounds by TLC technique on spot color observation both visually and irradiated by UV 254 nm and 365 nm, obtained Rf value presented in Table 3.

Table 1: Antibacterial activity result of *M.oleifera* L. leaf fractionation against Staphylococcus aureus and Escherichia coli

Sample concentration	Rf	Average diameter of inhibitory zone	
		Staphylococcus aureus	Escherichia Coli
Ethyl Acetate Faction 50mg/mL	0.28	9.21±3.72	7.1±1.9
	0.38	10.21±3.49	10.2±2.9
	0.49	8.77 ±3.19	10.6±1.1
	0.69	9.91 ±1.88	8.8±1.7
	0.79	10.17±1.11	10.7±1.8
	0.86	12.26±2.69	11.8±1.5
	0.94	13.73±0.29	14.5±0.7
Ethanol Fraction 50mg/mL	0.18	5.46 ±2.53	10.13±2.68
	0.33	8.34 ±3.05	9.39±2.08
	0.38	9.70 ±3.22	9.30±1.30
	0.75	7.67 ±5.20	10.06±2.54
	0.92	14 ±2.53	10.86±2.91
Erythromycin 15µg/*disk*		38.7±1.41	-
Chloramphenicol 30µg/disk		-	32.33±0.58

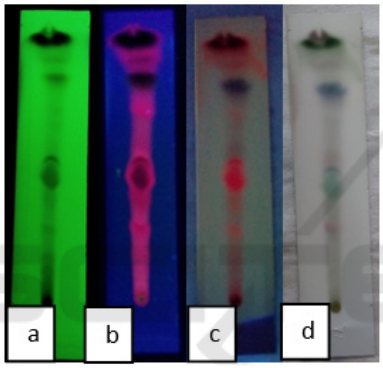

Figure 1: Identification of terpenoids compounds with TLC, (a) UV 254 nm (b) UV 365 nm (c) derivatization, UV 365 nm (d) derivatization, visual.

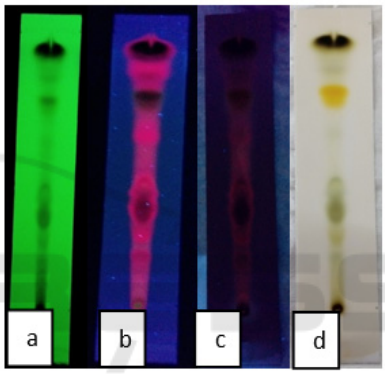

Figure 3: Identification of polyphenols compounds with TLC (a) UV 254 nm (b) UV 365 nm (c) derivatization, UV 365 nm (d) derivatization, visual.

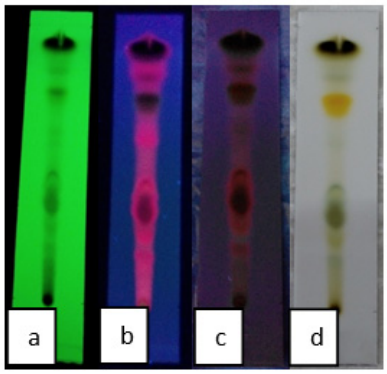

Figure 2: Identification of flavonoids compounds with TLC, (a) UV 254 nm (b) UV 365 nm (c) derivatization, UV 365 nm (d) derivatization, visual.

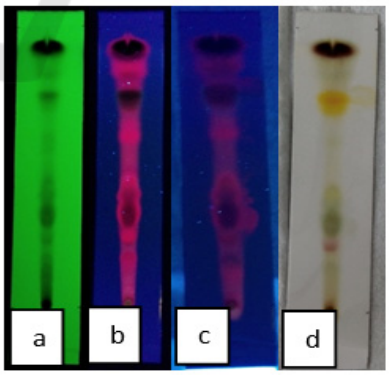

Figure 4: Identification of anthraquinone compounds with TLC (a) UV 254 nm (b) UV 365 nm (c) derivatization, UV 365 nm (d) derivatization, visual.

Table 2: TLC Results from the ethyl acetate fraction of M.oleifera leaves mobile phase N-Hexane : Ethyl Acetate (4: 6).

Rf	Flavonoids (10% Sulfuric Acid)	Terpenoid (Anisaldehyde-Sulfuric Acid)	Polyphenols ($FeCl_3$)	Anthraquinone (KOH 10% in methanol)
0.28	-	Purple red	-	-
0.38	-	-	-	Red purple
0.49	-	-	-	purple green
0.69	-	Purple red		-
0.79	Intensive yellow	-	-	-
0.86	-	Purple red	-	-
0.94	-	-	Black	Brownish yellow

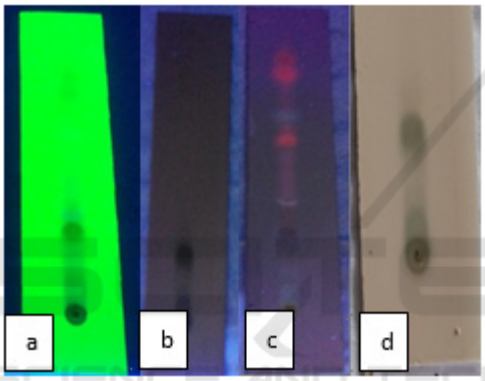

Figure 5: Identification of polyphenols compounds with TLC (a) UV 254 nm (b) UV 365 nm (c) derivatization, UV 365 nm (d) derivatization, visual.

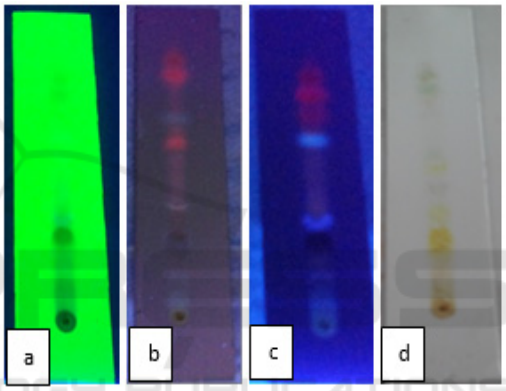

Figure 7: Identification of flavonoid compounds with TLC (a) UV 254 nm (b) UV 365 nm (c) derivatization, UV 365 nm (d) derivatization, visual.

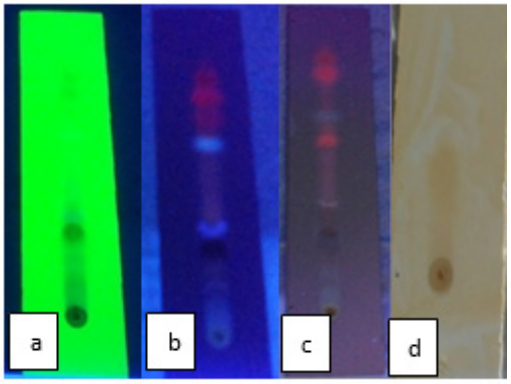

Figure 6: Identification of alkaloid compounds with TLC (a) UV 254 nm (b) UV 365 nm (c) derivatization, UV 365 nm (d) derivatization, visual.

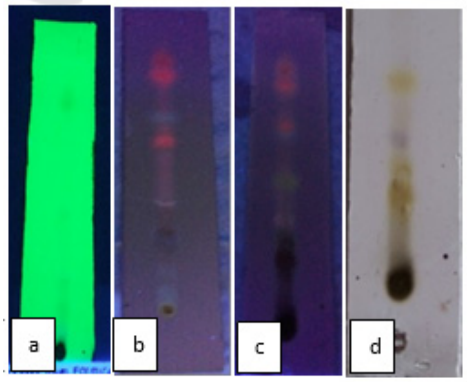

Figure 8: Identification of saponin compounds with TLC (a) UV 254 nm (b) UV 365 nm (c) derivatization, UV 365 nm (d) derivatization, visual.

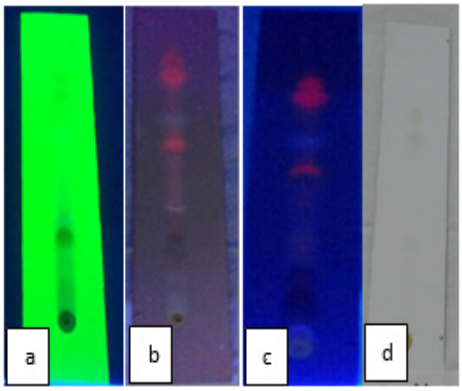

Figure 9: Identification of terpenoid compounds with TLC (a) UV 254 nm (b) UV 365 nm (c) derivatization, UV 365 nm (d) derivatization, visual.

Table 3. TLC results from the Ethole Acetate fraction of M.oleifera leaves mobile phase Ethyl acetate: n-Hexane: methanol (4: 0.5: 0.5) added 1 drop of formic acid.

Rf	Polyphenols ($FeCl_3$)	Alkaloids (Dragen-droff)	Saponin (Anisaldehyde-Sulfuric Acid)	Flavonoids (10% Sulfuric Acid)	Terpenoid (Anisaldehyde-Sulfuric Acid)
0,18	Black	Orange	-	-	-
0,33	-	-	-	-	-
0,38	-	-	purple	-	-
0,75	-	-	-	Yellow Intensive	-
0,92	-	-	-	-	purple

The secondary metabolite compounds detected in the ethyl acetate and ethanol fraction have antibacterial activity. The flavonoid antibacterial mechanism works by inhibiting the synthesis of DNA and RNA from bacteria. In Proteus vulgaris bacteria, flavonoids show a process of inhibiting the formation of strong bacterial DNA. Whereas in the process of inhibiting the formation of bacterial RNA the strongest results were found in S. aureus. In addition to inhibiting flavonoid DNA and RNA synthesis it also inhibits the formation of bacterial cytoplasmic membranes. Examples of antibacterial flavonoids are apigenin, quercetin, flavonone, isoflavones, luteolin, and derivatives of epigalotekin (Patel *et al.*, 2014).

The terpenoid compound as an antibacterial works by inhibiting bacterial growth through destruction in the bacterial cell membrane. In the polyphenol group, antibacterial activity works by binding to proteins, damaging cell membranes, and inhibiting the reverse transcriptase enzyme so that bacterial cells cannot be formed (Emmanuel *et al.*, 2014).

In this study also found the antrakinon group. In this group has a broad antibacterial activity. Anthraconone works by forming complexes with nucleophilic amino acids in proteins that can cause proteins to lose their function. Quinone reacts with cell hair adhesion proteins, cell wall polypeptides, and echoenzymes released through membranes (Putra, 2010).

Polyphenolic compounds can also have antibacterial activity. The mechanism of action of polyphenols as an antibacterial is by binding to proteins, damaging bacterial cell membranes and inhibiting enzyme expenditure. Inhibiting the reverse transcriptase enzyme (the reverse transcription process that is copying RNA into DNA) and DNA topoisomerase (curling) so that bacterial cells cannot be formed (Nuria, Faizatun and Sumantri, 2009).

Alkaloid compounds contain nitrogen groups and are usually present in high amounts in certain plant parts. This compound is usually found in seeds, fruit, leaves, roots and on the bark. One of the functions of alkaloids is as a poison to protect plants from animal and insect attacks, but some are used as treatments such as morphine and quinine. Alkaloid compounds can interfere with the formation of cross bridges of peptidoglycan compounds in bacterial cells so that the cell wall layer is not formed intact which then results in cell death (Patel *et al.*, 2014).

The mechanism of action of saponin as an antibacterial is that it can cause leakage of proteins and enzymes from within the cell. Saponins can be anti-bacterial because of their surface active substances which reduce the surface tension of bacterial cell walls and damage membrane

permebiality. Damage to the cell membrane is very disturbing survival of bacteria. Saponins diffuse through the outer membrane and cell walls of the vulnerable and then bind to the cytoplasmic membrane so that it interferes with and reduces the stability of the cell membrane (Kalpana, Moorthi and Kumara, 2013).

4 CONCLUSIONS

The compounds in the ethyl acetate fraction show the class of compounds Flavonoids, Terpenoids, Polyphenols and Anthraquinone while in the ethanol fraction *Moringa oleifera* L. leaves show the compounds of the compounds Alkaloids, Flavonoids, Terpenoids, Polyphenols, and Saponin while in the ethanol fraction *Moringa oleifera* L. Antimicrobial activity is shown in the ethyl acetate and ethanol fraction in both Staphylococcus aureus and Escherichia coli.

REFFERENCES

Abdallah, E. (2016) 'Antibacterial properties of leaf extracts of Moringa oleifera Lam. growing in Sudan', *Journal of Advances in Medical and Pharmaceutical Sciences*, 5(1), pp. 1–5. Available at: https://pdfs.semanticscholar.org/39bc/e9f1d9288cca16c1a0383ab5b62eb09d3e52.pdf (Accessed: 18 November 2019).

Akinyeye, A., Solanke, E. and Adebiyi, I. (2014) 'Phytochemical and antimicrobial evaluation of leaf and seed of Moringa olifera extracts', *Int. J. Res. In Med*, 4(6). Available at: https://pdfs.semanticscholar.org/f4ee/2b2d6aedc5f9ae865a89ea6d53a7b4ac1b3c.pdf (Accessed: 18 November 2019).

Brooks, G. F. *et al.* (2013) *Medical Microbiology*. 26th edn. New York: McGraw-Hill Companies.

Emmanuel, S. *et al.* (2014) 'Phytochemical and antimicrobial studies of methanol, ethyl acetate, and aqueous extracts of Moringa oleifera seeds', *American Journal of Ethnomedicine*, 1(5). Available at: https://www.researchgate.net/profile/Olajide_Olutayo/publication/332528284_Phytochemical_and_Antimicrobial_Studies_of_Methanol_Ethyl_acetate_and_Aqueous_Extracts_of_Moringa_oleifera_Seeds/links/5ae04180aca272fdaf8bd9b4/Phytochemical-and-Antimicrobial-Stud (Accessed: 19 November 2019).

Gyawali, R. and Ibrahim, S. A. (2014) 'Natural products as antimicrobial agents', *Food Control*. Elsevier, 46, pp. 412–429. doi: 10.1016/J.FOODCONT.2014.05.047.

Kalpana, S., Moorthi, S. and Kumara, S. (2013) 'Antimicrobial activity of different extracts of leaf of Moringa oleifera (Lam) against gram positive and gram negative bacteria', *International Journal of Current Microbiology*, 12(2).

Luqman, S. *et al.* (2012) 'Experimental Assessment of Moringa oleifera Leaf and Fruit for Its Antistress, Antioxidant, and Scavenging Potential Using In Vitro and In Vivo Assays.', *Evidence-based complementary and alternative medicine : eCAM*. Hindawi, 2012, p. 519084. doi: 10.1155/2012/519084.

Minaiyan, M. *et al.* (2014) 'Anti-inflammatory effect of Moringa oleifera Lam. seeds on acetic acid-induced acute colitis in rats.', *Avicenna journal of phytomedicine*. Mashhad University of Medical Sciences, 4(2), pp. 127–36. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25050310 (Accessed: 18 November 2019).

Moyo, B., Masika, P. and Muchenje, V. (2012) 'Antimicrobial activities of Moringa oleifera Lam leaf extracts', *AFRICAN JOURNAL OF BIOTECHNOLOGY*. Academic Journals (Kenya), 11(11), pp. 2797–2802. doi: 10.5897/AJB10.686.

Nuria, M., Faizatun, A. and Sumantri, S. (2009) 'Uji Aktivitas Antibakteri Ekstrak Etanol Daun Jarak Pagar (Jatropha Curcas L) Terhadap Bakteri Staphylococcus aureus ATCC 25923, Escherichia coli ATCC', *Mediagro*, 5(2). Available at: https://www.publikasiilmiah.unwahas.ac.id/index.php/Mediagro/article/download/559/680 (Accessed: 19 November 2019).

Oluduro, A. O. (2012) 'Evaluation of Antimicrobial properties and nutritional potentials of Moringa oleiferaLam.leaf in South-Western Nigeria', *Malaysian Journal of Microbiology*, 8(2), pp. 59–67.

Patel, P. *et al.* (2014) 'Phytochemical analysis and antifungal activity of Moringa oleifera', *Journal of Pharmacy*, 6(5). Available at: https://pdfs.semanticscholar.org/63c9/b2bd4b4dd79522ccfaeabf083bcb5a04be69.pdf (Accessed: 19 November 2019).

Putra, I. (2010) 'AKTIVITAS ANTIBAKTERI EKSTRAK KULIT BUAH MANGGIS (Garcinia mangostana L.) SERTA KANDUNGAN SENYAWA AKTIFNYA [Antibacterial Activity of', *Jurnal Teknologi Pangan dan Industri*, 21(1). Available at: http://journal.ipb.ac.id/index.php/jtip/article/view/2422 (Accessed: 19 November 2019).

Sarker, S. D., Latif, Z. and Gray, A. I. (2006) 'Natural Product Isolation', in *Natural Products Isolation*. Totowa, NJ: Humana Press, pp. 1–25. doi: 10.1385/1-59259-955-9:1.

Wikansari, (Nurvita) (2012) 'Pemeriksaan Total Kuman Udara Dan Staphylococcus Aureus Di Ruang Rawat Inap Rumah Sakit X Kota Semarang', *Jurnal Kesehatan Masyarakat Universitas Diponegoro*. Diponegoro University, 1(2), p. 18795. Available at: https://www.neliti.com/publications/18795/pemeriksaan-total-kuman-udara-dan-staphylococcus-aureus-di-ruang-rawat-inap-ruma (Accessed: 18 November 2019).

Yacob, T. *et al.* (2011) 'Resistensi Antibakteri pada Pasien Infeksi Saluran Kemih (ISK) dengan Kateterisasi Urin di Bagian Penyakit Dalam RSUD Arifin Achmad Pekanbaru', *Jurnal Ilmu Kedokteran*, 5(2), pp. 94–100.