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ANTIBACTERIAL ACTIVITY OF PURIFIED EXTRACT OF AFRICAN LEAVES (*Vernonia amygdalina Delile*) AGAINST *Escherichia coli*

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ABSTRACT
The African leaf (*Vernonia amygdalina* Delile) is an Indonesian plant used by the community as a traditional medicine. *V.amygdalina* contains active compounds such as flavonoids, tannins, saponins, and steroids which have antibacterial properties. This study aims to determine the antibacterial activity and the most effective concentration of purified extracts of *V.amygdalina* against the Escherichia coli bacteria. *V.amygdalina* simplicia was extracted using the maceration method. Ethanolic extract *V.amygdalina* was tested for the quality of the extract parameters and purified. Then, the purified extract *V.amygdalina* was tested for phytochemical screening and antibacterial activity against Escherichia coli bacteria by well-diffusion with concentrations of 30%, 40%, and 50%. The result of the quality parameter test for ethanolic extract *V.amygdalina* meets the requirements of specific and non-specific parameters. Purified extract *V.amygdalina* positively contains flavonoids, tannins, saponins, and steroids. Antibacterial activity of 0.05% chloramphenicol and purified extract *V.amygdalina* concentrations of 30%, 40%, and 50% against Escherichia coli bacteria had inhibition of 23.42 mm, 8.40± 0.315 mm, 9.44± 0.543 mm, and 11.59 ± 1,640 mm. The produced most effective inhibition was at a concentration of 50%. The results showed that the purified extract of *V.amygdalina* has antibacterial activity in the moderate to strong category, and the most effective concentration against *Escherichia coli* bacteria is the concentration of 50%.

Keywords: African leaves; Purified extract; Antibacterial activity

1. INTRODUCTION

Plants in Indonesia have many uses in people's lives, one of which is used in traditional medicine. Traditional medicine is a concoction of natural ingredients or preparations that can come from plants, minerals, animals, or galenica preparations, or a mixture of ingredients that have been used for generations as a healing medium that can be applied according to the norms prevailing in that society. The advantage of the basic ingredients of a medicinal plant is that the side effects produced are relatively low (Isnawati et al., 2019). One of the plants that can be used in traditional medicine is African leaves. This leaf is better known as the leaf of a thousand diseases, namely a leaf that people believe can be efficacious in treating various diseases (M. Muhammad et al., 2023). African leaves have anti-diabetic activity (Yunitasari et al., 2022), antioxidant (Karlina et al., 2023), treat cholesterol, headaches, fever, throat infections, and cancer, treat malaria, heal wounds, and coughs, are antibacterial, and treat digestive problems (Hasibuan

et al., 2024; Oyeyemi et al., 2018). The various properties of various medicinal plants can be caused by the presence of secondary metabolite compounds (Yunitasari et al., 2022). Based on research, the secondary metabolites found in African leaf plants are flavonoids, alkaloids, terpenoids, glycosides, tannins, and saponins (Ogidi et al., 2019).

Indonesia has a tropical climate, which makes bacteria grow easily, one of which is a type of bacteria that is pathogenic (disease). One example of pathogenic bacteria is Escherichia coli bacteria. This bacterium belongs to the enterobacteria group of gram-negative bacteria which are often found in human digestion, animal digestion, soil, water, and air (Booth, 2018). Apart from that, Escherichia coli is also often found in the human digestive tract such as the large intestine as a microorganism or normal flora which can cause large amounts of salt and water to be lost from the body. Thus, pathogenic bacteria such as Escherichia coli can be the main cause of diarrhea (Riley, 2020). Research on antibacterial tests on African leaves showed that the ethanolic extract of African leaves on the proliferation of Escherichia coli bacteria could provide inhibitory power at an extract concentration of 50% (Habtom & Gebrehiwot, 2019).

The purified extract is an extract that is free from various components of ballast substances (impurity compounds contained in the sample) which can disrupt a matrix (marker compound) in natural materials, disrupting the resulting biological activity (Carolia & Noventi, 2016). The extract purification process aims to obtain various pure extract components that are free from other chemical components that are not needed in the extract such as wax, plasticizer, and fat (Ramadhani & Novema, 2022), in this research purification of the extract was carried out to maintain some of the chemical contents of the extract which have a synergistic effect and can maximize activity in the treatment process. Therefore, this study aims to determine the activity of purified extract of African leaves (*Vernonia amygdalina* Delile) against *Escherichia coli* bacteria.

2. METHODS

2.1. Plant Materials

African leaves obtained from Astana Village, Gunung Jati, Cirebon Regency were collected first. Then, plant determination is carried out to ensure the correctness of the plant parts and types of African plants that will be used in the research. The plant determination process was carried out at the Plant Taxonomy Laboratory, Biology Department, FMIPA, Padjadjaran University. The results of the African Leaf determination are based on the plant determination certificate Number 28/HB/04/2023.

2.2. Preparation of the Extracts

African Simplicia leaf powder is extracted using the maceration method. A total of 600 grams of simplicia powder was put into a closed glass vessel. Add 4.5 L (75 parts) filter liquid in the form of 96% ethanol, cover, and let sit for 3 days, stirring occasionally. After 3 days, the solution was filtered and squeezed, the dregs were washed with 1.5 L (25 parts) filter fluid until a filtrate was obtained. Then cover the container, and leave it in a cool place protected from light for 2 days. After that, concentrate using a rotary evaporator at 40 °C to one-third. Continue evaporation with an electric water bath until a thick extract is obtained (Karlina et al., 2023). Specific parameter testing of African leaf ethanolic extract includes organoleptic tests (odor, texture, and color), water-soluble extract, and ethanol-soluble extract (Kunaedi et al., 2023) while non-specific parameter tests include water content, total ash, acid-insoluble ash, and drying shrinkage test (Depkes RI, 2017). The ethanolic extract of African leaves was purified using n-hexane and distilled water (1:1) (Iryani et al., 2021).

2.3. Qualitative Analysis of Phytochemical Screening

Phytochemical screening includes tests for alkaloids, flavonoids, saponins, tannins, steroids, or triterpenoids. The purified extract of African leaves which had been diluted with distilled water was tested for flavonoids by adding 2 mg of magnesium powder and 3 drops of concentrated

hydrochloric acid. Positive results indicate the appearance of an orange color. Alkaloid test by adding 2-3 drops of Mayer and Dragendorf reagent (Putri et al., 2021). The result is positive if a yellowish-white precipitate forms with Mayer's reagent and a brownish-red precipitate forms with Dragendorf's reagent (Ladeska & Dingga, 2019). The saponin test is carried out by adding 10 mL of hot water, then cooling and shaking vigorously. The presence of saponin If a firm and stable foam forms for no less than 10 seconds and does not disappear when 1 drop of 2N hydrochloric acid is added. tannin test by adding 2-3 drops of 1% iron (III) chloride (Indriaty et al., 2023). The result is positive if a blackish-green color forms. In the steroid or Triterpenoid Test by adding 1 drop of glacial acetic acid and 2 drops of concentrated sulfuric acid, the results obtained are green, indicating the presence of steroids, if a purple color is formed, it indicates the presence of triterpenoids.

2.4. Preparation of Culture Media and Sterilization

2.4.1. Sterilization

The tools to be used are washed thoroughly using detergent and dried. After that, certain tools are covered on top with fatty cotton wrapped in gauze, then wrapped in parchment paper, and tied with mattress thread. For glass beakers, the mouth is only covered with parchment paper and tied with mattress thread, while Petri dishes are only wrapped with parchment. Sterilize using an autoclave at 121°C for 15 minutes.

2.4.2. Nutrient Agar Media

Media in Petri Dishes - Weigh the nutrients so that they are 1.6 grams. Dissolve 80 mL of distilled water in an Erlenmayer, then stir until dissolved. Heat the nutrient agar solution over low heat until clear and homogeneous. Cover with greased cotton. Then wrap it in parchment paper and tie it with mattress twine. Sterilize using an autoclave at 121 °C for 15 minutes.

The Media is Tilted - Weigh the nutrient so that it weighs 0.16 grams. Dissolve with 8 mL of distilled water in an Erlenmeyer flask, and stir until dissolved. Heat the nutrient agar solution over low heat until clear and homogeneous. Then pour the agar medium into a test tube. Sterilize by autoclaving at 121 °C for 15 minutes. After that, tilt it at 10° and let the media solidify (Balouiri et al., 2016).

Bacterial Rejuvenation - Take the ose needle in the flambir until the tip of the needle becomes incandescent, do this 3 times. Then take the Escherichia coli bacteria from the test tube, open the tube cap, and flammable the mouth of the tube. Take 1 dose of Escherichia coli bacteria, then flammable the mouth of the tube and close it again. Inoculate on agar media slanted in a zig-zag manner, cover, and incubate at 37 °C for 24 hours (Chavez-Esquivel et al., 2021).

Bacterial Suspension - Take 3 mL of 0.9% Sodium Chloride using a syringe, then put it in a test tube. Take 3 inocula of Escherichia coli bacteria, then put them in a test tube filled with 0.9% Sodium Chloride. Then the turbidity is seen by comparing the standard turbidity Mc. Farland (Balouiri et al., 2016).

2.5. Antimicrobial Activities

A total of 1 mL of bacterial suspension was inoculated into 80 mL of warm Nutrient Agar media, and shaken gently. The nutrient media is poured into 3 petri dishes of 20 mL each, then wait for 15 minutes (Indriaty et al., 2022). Before printing the holes, first mark the surface of the area at the back of the petri dish with a marker (A = positive control, B = negative control, C=30% concentration of purified extract of African leaves, D=40% and E=50%) (Héjja et al., 2024). After marking, print a hole using a perforator with a diameter of 6 mm which has previously been flammable. Then, the holes in the media were filled with purified extract of African leaves according to the predetermined concentration, positive control, and negative control 20 µl each using a micropipette. After that, leave it for 2 hours, then put it in an incubator at a temperature of 35-37 °C for 18-24 hours. The resulting clear zone around the hole is observed and measured using a caliper (Jarriyawattanachaikul et al., 2016; Karimi et al., 2018).

3. RESULTS AND DISCUSSION

Plant determination carried out at the Plant Taxonomy Laboratory, Department of Biology, FMIPA, Padjadjaran University, Bandung Number 28/HB/04/2023 showed that the plant part used for research was correct, African leaves from the Asteraceae tribe with the species *Vernonia amygdalina* Delile. The organoleptic test results on African leaf Simplicia have a dark green color with a distinctive, aromatic, and quite pungent odor. The resulting texture is a dry, coarse powder. The organoleptic test results of the ethanolic extract of African leaves have a dark green color, a distinctive aromatic odor that is not too strong and the resulting texture is thick like a paste. Extract standardization testing is carried out to determine the quality parameters of the extract contained in an extract under study by established standards. The results of testing extract quality parameters are in the **Table 1**.

Table 1. Value of specific parameters and non-specific parameters of ethanolic extract

Parameters	Mean±SD %	Standard
Water Content	7.53±0.0055	<12.5%
Water Soluble Extract	17.20±0.0006	>12%
Ethanol Soluble Extract	31.49±0.0055	>8%
Total Ash	6.30±0.0281	<10.2%
Acid-insoluble ash	0.28±0.0015	<0.6%
Dry Shinkage	5.09±0.0052	<10%

The results of the phytochemical screening test for purified extracts of African leaves were positive for containing active compounds of flavonoids, saponins, tannins, and steroids. Based on the inhibition zone classification, the antibacterial activity test of the purified extract of African leaves has a clear zone in the moderate to strong category (Sanam et al., 2022). At concentrations of 30%, 40%, and 50%, it has antibacterial activity against Escherichia coli bacteria with the resulting inhibitory power being 8.40 ± 0.315 mm; 9.44 ± 0.543 mm; and 11.59 ± 1,640 mm. The most effective inhibitory power of the purified extract of African leaves is at a concentration of 50% with an average inhibitory power of 11.59 ± 1,640 mm. Antibacterial activity of purified extract of African leaves against Escherichia coli bacteria in **Figure 1**.

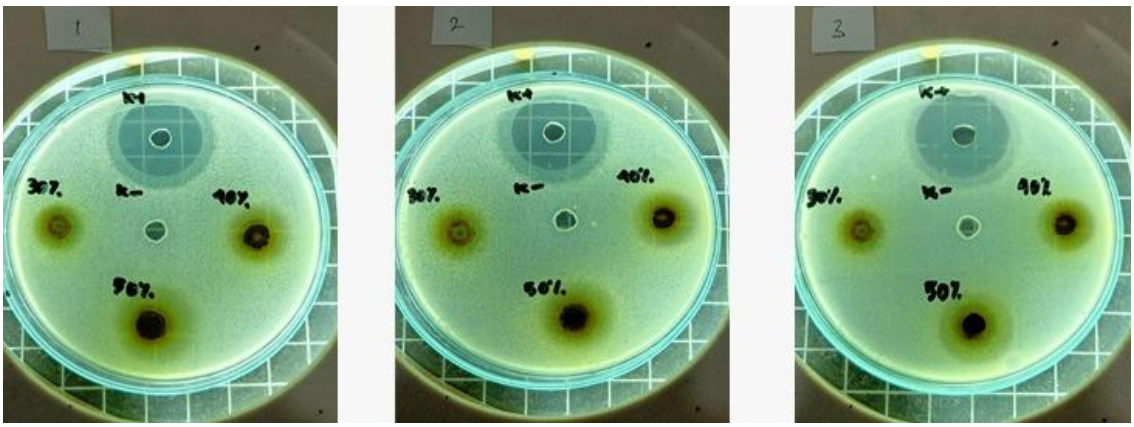

Figure 1. Antibacterial activity of purified extract of african leaves against *Escherichia coli*

Based on the data obtained, it shows that the ethanolic extract of African leaves has met various extract quality parameter requirements including water content, water-soluble essence content, ethanol-soluble essence content, total ash content, acid-insoluble ash content, and drying loss. Standardization of extract water content in African leaf extract was carried out using a tool in the form of MAB (Moisture Analyzer Balance). The water content results obtained from research that has been carried out are 7.53±0.0055%. The requirements for good extract quality parameters for African leaf extract according to the (Depkes RI, 2017) are no more than 12.5%.

This shows that the ethanolic extract of African leaves has water content that meets the requirements, the result of which is no more than 12.5%. The purpose of determining the water content in extracts is to provide a minimum limit or range for the amount of water content in a sample, where the higher the water content in an extract, the easier it is for fungi or mold to grow, thereby reducing the biological activity of the extract during the storage period.

Testing the levels of water-soluble essence and the levels of ethanol-soluble essence aims to determine the number of compounds dissolved in water (polar) and ethanol (semi-polar-non-polar in nature). In this study, the results of the water-soluble essence from African leaf extract were 17.20±0.0006%, while the ethanol-soluble essence was 31.49±0.0055%. The quality parameter requirements for water-soluble essence content and ethanol-soluble essence content are that the water-soluble essence content is not less than 12% and the ethanol-soluble essence content is not less than 8%. This shows that the extract quality parameters of African leaf extract are more soluble in ethanol than in water because African leaf extract has active compounds that are more likely to be easily dissolved in ethanol than water. After all, ethanol is a universal solvent so it can attract polar compounds and non-polar while water is only able to attract polar compounds.

The ash content testing carried out included total ash content with the results obtained in the research carried out being 6.30±0.0281%, while the acid-insoluble ash content was 0.28±0.0015%. According to the (Depkes RI, 2017), the requirement for a good total ash content for African leaf ethanolic extract is no more than 10.2%, while the acid-insoluble ash content is no more than 0.6%, so testing the ash content of the leaf ethanolic extract Africa has met the requirements. Determination of total ash content is carried out to determine the amount of internal and external mineral content remaining after the washing process which can come from the beginning to the end of the extract-making process. Meanwhile, determining the acid-insoluble ash content aims to evaluate the extract for contamination by materials containing silica such as soil and sand contained in the extract studied. Drying loss in testing the quality parameters of African leaf extract which was carried out was 5.09±0.0052%. The requirement for good extract drying shrinkage is <10%. Determination of drying loss is carried out to show how many compounds contained in the extract have been lost or easily evaporated during the drying process. Drying loss is a parameter for an extract to maintain quality to avoid fungal growth (Utami et al., 2022).

Based on research data from antibacterial activity tests, shows that all the wells in the samples containing purified extracts of African leaves have clear zones in the medium to strong category. The inhibitory power produced by the purified extract of African leaves is due to the secondary metabolite compounds contained in the purified extract of African leaves. These metabolite compounds include flavonoids, saponins, tannins, and steroids. Flavonoids act as antibacterials by denaturing bacterial cell proteins, damaging bacterial cell membranes, and inhibiting energy metabolism in bacteria so that bacterial growth will be hampered and disrupted (Gutiérrez-Venegas et al., 2019). Saponin has a role as an antibacterial with its mechanism of action, namely reacting with porins (trans-membrane proteins) on the outer membrane of bacterial cell walls, forming strong polymer bonds resulting in damage to porins which are the gates for the entry and exit of compounds which will reduce the permeability of bacterial cell membranes and result in cell Bacteria will experience a lack of nutrition, so that bacterial growth is hampered or dies (Alina et al., 2023). Then, tannins can also act as antibacterials by binding to the proteins of bacteria by contracting the cell walls so that permeability and cell wall formation will be hampered. Steroids can act as antibacterials whose mechanism of action is to interact with the phospholipid membrane of bacterial cells which are permeable to lipophilic compounds this causes the integrity of the cell membrane to decrease and the morphology of the cell membrane to change into cells that can easily become brittle and lyse (Othman et al., 2019).

4. CONCLUSION

A purified extract of African leaves (*Vernonia amygdalina* Delile) at concentrations of 30%, 40% and 50% has antibacterial activity against Eschericia coli bacteria with the resulting inhibitory power of 8.40 ± 0.315 mm; 9.44 ± 0.543 mm; and 11.59 ± 1.640 mm. The most effective inhibitory power of the purified extract of African leaves is at a concentration of 50% with an average inhibitory power of 11.59 ± 1.640 mm. The higher the concentration of purified extract of African leaves, the greater the inhibitory power of bacterial growth due to the increasing content of bioactive bacteria inhibitors. Further research is needed on African leaves using other extraction methods and bacteria.

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Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Competing Interests

The authors declare no competing interest.

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7. REFERENCES

Alina, P., Kiełb Ewa, P., & František, Z. (2023). Antimicrobial activity of saponin-containing plants: review. *Journal of Dairy, Veterinary & Animal Research*, *12*(2), 121–127. https://doi.org/10.15406/jdvar.2023.12.00336

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Booth, M. (2018). Climate Change and the Neglected Tropical Diseases. In *Advances in Parasitology* (1st ed., pp. 39–126). https://doi.org/10.1016/bs.apar.2018.02.001

Carolia, N., & Noventi, W. (2016). Potensi Ekstrak Daun Sirih Hijau (Piper betle L .) sebagai Alternatif Terapi Acne vulgaris. *Studi Pendidikan Dokter Fakultas Kedokteran Universitas Lampung*, *5*(1), 140–145.

Chavez-Esquivel, G., Cervantes-Cuevas, H., Ybieta-Olvera, L. F., Castañeda Briones, M. T., Acosta, D., & Cabello, J. (2021). Antimicrobial activity of graphite oxide doped with silver against Bacillus subtilis, Candida albicans, Escherichia coli, and Staphylococcus aureus by agar well diffusion test: Synthesis and characterization. *Materials Science and Engineering: C*, *123*, 111934. https://doi.org/10.1016/j.msec.2021.111934

Depkes RI. (2017). *Farmakope Herbal Indonesia* (2nd ed.). Departemen Kesehatan Republik Indonesia.

Gutiérrez-Venegas, G., Gómez-Mora, J. A., Meraz-Rodríguez, M. A., Flores-Sánchez, M. A., & Ortiz-Miranda, L. F. (2019). Effect of flavonoids on antimicrobial activity of

microorganisms present in dental plaque. *Heliyon*, *5*(12), e03013. https://doi.org/10.1016/j.heliyon.2019.e03013

Habtom, S., & Gebrehiwot, S. (2019). In vitro Antimicrobial Activities of Crude Extracts of Vernonia amygdalina and Croton macrostachyus against Some Bacterial and Fungal Test Pathogens. *The Journal of Phytopharmacology*, *8*(2), 57–62. https://doi.org/10.31254/phyto.2019.8206

Hasibuan, P. A. Z., Keliat, J. M., Lubis, M. F., & Nasution, A. (2024). The ethyl acetate extract of Vernonia amygdalina leaf ameliorates gemcitabine effect against migration and invasion of PANC-1 cells via down-regulation the VEGF, COX2, and RAS/MEK pathways. *Saudi Pharmaceutical Journal*, *32*(1), 101872. https://doi.org/10.1016/j.jsps.2023.101872

Héjja, M., Mihok, E., Alaya, A., Jolji, M., György, É., Meszaros, N., Turcus, V., Oláh, N. K., & Máthé, E. (2024). Specific Antimicrobial Activities Revealed by Comparative Evaluation of Selected Gemmotherapy Extracts. *Antibiotics*, *13*(2), 181. https://doi.org/10.3390/antibiotics13020181

Indriaty, S., Indawati, Ii., Hidayati, N. R., Karlina, N., Nurlia, S., & Firmansyah, D. (2023). Formulasi Dan Uji Stabilitas Masker Gel Peel-Off Ekstrak Etanol Daun Belimbing Wuluh Dengan Variasi Konsentrasi PVA. *Medical Sains : Jurnal Ilmiah Kefarmasian*, *8*(2), 797–810. https://doi.org/10.37874/ms.v8i2.764

Indriaty, S., Karlina, N., Hidayati, N. R., Firmansyah, D., Senja, R. Y., & Zahiyah, Y. (2022). Formulasi Dan Uji Aktivitas Deodoran Spray Ekstrak Etanol Herba Kemangi Terhadap Bakteri Staphylococcus aureus. *Medical Sains : Jurnal Ilmiah Kefarmasian*, *7*(4), 973–982. https://doi.org/10.37874/ms.v7i4.566

Iryani, Y. D., Astuti, I. Y., & Diniatik, D. (2021). Optimasi Formula Sediaan Losion Tabir Surya dari Ekstrak Etanol Terpurifikasi Kulit Buah Manggis (Garcinia mangostana L) Dengan Metode Simplex Lattice Design. *Jurnal Sains Farmasi & Klinis*, *8*(2), 145. https://doi.org/10.25077/jsfk.8.2.145-156.2021

Isnawati, A., Gitawati, R., Raini, M., Alegantina, S., & Setiawaty, V. (2019). Indonesia basic health survey: self-medication profile for diarrhea with traditional medicine. *African Health Sciences*, *19*(3), 2365–2371. https://doi.org/10.4314/ahs.v19i3.9

Jarriyawattanachaikul, W., Chaveerach, P., & Chokesajjawatee, N. (2016). Antimicrobial Activity of Thai-herbal Plants against Food-borne Pathogens E. Coli, S. Aureus and C. Jejuni. *Agriculture and Agricultural Science Procedia*, *11*, 20–24. https://doi.org/10.1016/j.aaspro.2016.12.004

Karimi, S., Azizi, F., Nayeb-Aghaee, M., & Mahmoodnia, L. (2018). The antimicrobial activity of probiotic bacteria Escherichia coli isolated from different natural sources against hemorrhagic E. coli O157:H7. *Electronic Physician*, *10*(3), 6548–6553. https://doi.org/10.19082/6548

Karlina, N., Kunaedi, A., Ahidin, D., Jannah, U., & Zahiyah, Y. (2023). Antioxidant Activity Test of African Leaves Purification Extract (Vernonia amygdalina Del) with DPPH Method. *Jurnal Farmasi Sains Dan Praktis*, *9*(1), 1–10. https://doi.org/10.31603/pharmacy.v9i1.7912

Kunaedi, A., Indawati, I., Karlina, N., Fadhillah, Z., Cantika, C. D., & Khulfiah, A. A. (2023). Study on the Optimization of Mulberry Leaf Extract By Macerating Ethanol and Microwave Assisted Extraction Method (Mae) With Natural Deep Eutectic Solvents (Nades). *Jurnal Farmasi Sains Dan Praktis*, *9*(1), 11–19. https://doi.org/10.31603/pharmacy.v9i1.7915

Ladeska, V., & Dingga, M. (2019). Kajian Farmakognosi dan Penetapan Kadar Flavonoid Total Herba Nanas Kerang (Tradescantia spathacea Sw.). *Jurnal Sains Farmasi & Klinis*, *6*(3), 254. https://doi.org/10.25077/jsfk.6.3.254-264.2019

M. Muhammad, E.D. Putra, H. Cintya, & D. Satria. (2023). The Effect of Solvent Towards Antioxidant Activity of Vernonia amygdalina Delile Leaves. *RASAYAN Journal of Chemistry*, *16*(02), 760–765. https://doi.org/10.31788/RJC.2023.1628059

Ogidi, O. I., George, D. G., & Esie, N. G. (2019). Ethnopharmacological properties of Vernonia amygdalina (Bitter Leave) medicinal plant Odangowei I Ogidi, Dienize G George and Ngozi G Esie. *Journal of Medicinal Plants Studies*, *7*(2), 175–181.

Othman, L., Sleiman, A., & Abdel-Massih, R. M. (2019). Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.00911

Oyeyemi, I. T., Akinlabi, A. A., Adewumi, A., Aleshinloye, A. O., & Oyeyemi, O. T. (2018). Vernonia amygdalina : A folkloric herb with anthelminthic properties. *Beni-Suef University Journal of Basic and Applied Sciences*, *7*(1), 43–49. https://doi.org/10.1016/j.bjbas.2017.07.007

Putri, A. N., Saputri, R., Astuti, K. I., Sari, I. P., & Saif Sulaiman, T. N. (2021). Specific and Non-Specific Parameters Standardization of Ethanolic 96% Extract of Kersen Leaves (Muntingia calabura L.). *Pharmacognosy Journal*, *13*(6s), 1710–1714. https://doi.org/10.5530/pj.2021.13.220

Ramadhani, M. A., & Novema, A. P. (2022). Aktivitas antibakteri ekstrak kasar dan terpurifikasi daun cengkeh (Syzygium aromaticum) terhadap Escherichia coli dan Staphylococcus aureus. *Borobudur Pharmacy Review*, *2*(1), 8–14. https://doi.org/10.31603/bphr.v2i1.6934

Riley, L. W. (2020). Distinguishing Pathovars from Nonpathovars: Escherichia coli. *Microbiology Spectrum*, *8*(4). https://doi.org/10.1128/microbiolspec.AME-0014-2020

Sanam, M., Detha, A., & Rohi, N. (2022). Detection of antibacterial activity of lactic acid bacteria, isolated from Sumba mare's milk, against Bacillus cereus, Staphylococcus aureus, and Escherichia coli. *Journal of Advanced Veterinary and Animal Research*, *9*(1), 53. https://doi.org/10.5455/javar.2022.i568

Utami, N. F., Elya, B., Hayun, & Kusmardi. (2022). Measurement of quality non-specific and specific-parameters of 70% ethanol extract and simplicia from cascara coffee robusta (Coffea canephora L.) and its potency as antioxidant. *IOP Conference Series: Earth and Environmental Science*, *1116*(1), 012008. https://doi.org/10.1088/1755-1315/1116/1/012008

Yunitasari, N., Swasono, R. T., Pranowo, H. D., & Raharjo, T. J. (2022). Phytochemical screening and metabolomic approach based on Fourier transform infrared (FTIR): Identification of α-amylase inhibitor metabolites in Vernonia amygdalina leaves. *Journal of Saudi Chemical Society*, *26*(6), 101540. https://doi.org/10.1016/j.jscs.2022.101540