JPPIPA 9(10) (2023)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Phytochemical Screening and Antibacterial Activity Test of Sumbawa white honey against *Bacillus megaterium*

Nurmi Hasbi[1*], Rosyunita[1], Adelia Riezka Rahim[2], Eusthacius Hagni Wardoyo[1], Ida Ayu Arnawati[3], Saskia Safarina Haza[3], Lale Nandhita Hulfifa[3], Abiel Dwi Cahya Firdaus Alamsyah[3], I Komang Satya Validika[3]

[1]Departemen of Microbiology, Faculty of Medicine, University of Mataram,
[2]Departemen of Parasitology Faculty of Medicine, University of Mataram,
[3]Medical Student Study Program Medical, Faculty of Medicine, University of Mataram

Received: August 6, 2023
Revised: September 4, 2023
Accepted: October 25, 2023
Published: October 31, 2023

Corresponding Author:
Nurmi Hasbi
nurmihasbi@unram.ac.id

DOI: 10.29303/jppipa.v9i10.4913

Abstract: *Bacillus megaterium* is a bacteria that causes human infections, causing keratitis, skin infections, brain abscesses and tissue infections. Treatment often uses chemical antibiotics. However, long-term antibiotics can cause new health problems such as resistance. Therefore, antibacterial ingredients are needed that can not only inhibit the growth of bacteria but also do not have a negative impact on health such as honey. Honey is thick, it can cure various diseases such as gastrointestinal, stomach, skin diseases, acute respiratory infections and coughs. This research aims to determine the ability of Sumbawa white honey as an antibacterial against *B. megaterium*. The method used in this research is paper disc diffusion. There were five treatments used in this study, between 100%, 75% and 25% honey concentrations, DMSO negative control and chloramphenicol positive control with five replications each. The results of the antibacterial activity test showed that the 100% concentration had an inhibitory zone diameter of 2.59 mm, then the 75% concentration was 1.46 while the 25% concentration was 0 mm. When compared with positive control, white honey was lower antibacterial. If it is in the white honey category, the inhibition zone category is included in the lower category.

Keywords: antibacterial; *Bacillus megaterium*; white honey sumbawa

Introduction

Infectious diseases are still a major health problem in several countries, especially in developing countries. The cause of infection is caused by a number of microorganisms such as pathogenic bacteria commonly known as disease germs. Bacterial infections have a major impact on human health. Disease can occur anywhere in the body and can be caused by the organism itself or by the body's response to its presence (ICON, 2023)(Bacterial infections can be transmitted by various mechanisms. The bacteria are transmitted to humans by air, water, food or live vectors. Infection-causing bacteria have characteristics that allow them to escape the human body's defense system. So that finally the virulence of these bacteria can attack the human immune system (Doron, S; Gorbach, 2020).

Bacillus megaterium is a bacterium that is reported to be able to infect humans. Infection due to *B. megaterium* is still rare, because only five cases have been reported in the world, but when infection occurs, the bacteria can become a serious health problem.*B. megaterium* is a Gram-positive, facultative anaerobic bacterium, which is usually found in natural environments such as soil, water, and air. Here are some cases of infection caused by *B. megaterium*. Keratitis is an infection of the cornea of the eye. In 2006, Ramos-Esteban JC *et al* reported the case of a 23-year-old man who developed late-onset lamellar keratitis caused by *B. megaterium* following a laser-assisted in situ keratomileusis (LASIK) procedure.

How to Cite:
Hasbi, N., Rosyunita, R., Rahim, A. R., Wardoyo, E. H., Arnawati, I. A., Haza, S. S., Hulfifa, L. N., Alamsyah, A. D. C. F., & Validika, I. K. S. (2023). Phytochemical Screening and Antibacterial Activity Test of Sumbawa white honey against Bacillus megaterium. *Jurnal Penelitian Pendidikan IPA*, *9*(10), 8707–8712. https://doi.org/10.29303/jppipa.v9i10.4913

Primary skin infection case (Duncan and Smith, (2011) reported the case of a 25-year-old woman with a primary skin infection caused by *B. megaterium*. Primary skin infections occur when bacteria infect the skin through cuts or abrasions on the surface of the skin. Brain abscess cases: Brain abscess infection is a serious condition in which pockets of pus form in the brain. Guo *et al*., (2015) reported the case of a woman with a brain abscess infection caused by *B. megaterium*. Pleural effusion cases. In the case reported by Crisafulli *et al*., (2019) a man was hospitalized with a pleural effusion that was ultimately caused by *B. megaterium*. Pleural effusion is the accumulation of excess fluid between the two layers of the pleural membrane in the lungs. The results of a study by Bocchi *et al*., (2020) reported an infection due to *B. megaterium* in the soft tissues of a 60-year-old man. Until now, information about *B. megaterium* and reported cases of infection may have been limited due to the rarity of cases. However, it is important to remain alert to the possibility of this infection, especially for high-risk individuals, such as those who have had invasive procedures or are immunocompromised. Therefore it remains relevant to detect this infection early, appropriate treatment to avoid the spread of *B. megaterium* bacteria.

Medical treatment of infectious diseases caused by bacteria is usually by taking drugs containing the right antibiotics and treating antiseptics properly. However, the use of antibiotics in the long term can cause new problems for health such as impaired liver function, decreased white blood cell count, the emergence of allergies and can also cause resistance so that disease treatment requires higher doses of antibiotics (Doron, S; Gorbach, 2020). Therefore, antibacterials are needed which can not only inhibit the growth of bacteria but also which do not have a negative impact on health, namely by using natural ingredients, one of which is by giving honey.

Honey is a thick, sweet-tasting liquid produced by bees from flower nectar and is thought to be efficacious for curing many diseases, such as digestive tract, stomach, skin diseases, acute respiratory infections, and coughs, as well as eye disorders. The ability of honey as an antibacterial is thought to have several components including sugar content; polyphenolic compounds; hydrogen peroxide; 1,2- dicarbonyl compounds; and bee defense-1. All of these elements are present at different concentrations depending on the nectar source, type of bee, and storage. These components work synergistically, enabling honey to fight a wide range of microorganisms including multi-drug resistant bacteria and modulate their resistance to antimicrobial agents. The effectiveness and potential of honey against microorganisms depends on the type of honey produced, which depends on its plant origin, bee health, processing and geographical origin. (Almasaudi, 2021).

Sumbawa is one of the honey-producing areas in Indonesia, one type of honey produced is Sumbawa white honey produced by *Apis mellifera*. These bees can be found around Mount Tambora, in Dompu Regency. Sumbawa white honey produced by *Apis mellifera* also has very good benefits, the same as other Sumbawa honey, such as Sumbawa black honey produced by *Trigona* spp. bees (Manguntungi *et al*., 2021) White honey was reported to have the same activity as black honey against the growth of pathogenic bacteria *Methicillin Resistant Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa Multi Resistant* (PaMR) (Rostinawati, 2009). Then LAB isolates from Sumbawa white honey were also reported as antibacterial against *S. thyposa, S. aureus, E. coli, E. ludwigii* and *L. adecarboxylata* bacteria (Manguntungi *et al*., 2021) Based on some of the literature above by looking at the potential of white honey as an antibacterial candidate derived from nature. The author plans to conduct a study to test the antibacterial concentration of *Apis melifera* bee white honey from Sumbawa against *B. megaterium* bacteria.

Method

Research Design

This study used a laboratory analytic experimental method with a Randomized Controlled Trial (RCT) type of post test design. In order to ensure that the samples and independent variables are randomized to the maximum, the study uses a single blinding technique, so that the researchers do not know the allocation of independent variables in each sample.

Time and Place Research

This research was conducted from May to July 2023 at the Pharmacology Laboratory and Microbiology Laboratory, Faculty of Medicine, Mataram University

Research Sample

The samples used in this study were *Bacillus megaterium* from Balai Laboratorium Kesehatan Pengujian dan Kalibrasi Provinsi Nusa Tenggara Barat and Sumbawa White Honey from the Tambora Mountain region, Sumbawa Island, West Nusa Tenggara.

Research Variable
Independent Variable

The independent variables used in this study are: (1) Concentration of honey in percentage: 25%, 75% and 100%; (2) Negative control: DMSO; (3) Positive control: Antibiotic chloramphenicol disc

Dependent Variable

The dependent variable used in this study was *B. megaterium* using the diffusion inhibition zone diameter method.

Tools and materials used

The tools used in this study were petri dishes, incubators, test tubes, scales, laminar air flow, loops, tweezers, labels, rulers, Bunsen, autoclaves, and paper discs. The materials used in this study were Sumbawa white honey, Blood Agar Plate (BAP) media, Mueller Hinton Agar (MHA) media, DMSO, alcohol, NaCl and standard Mcfarland 0.5 solution**.**

Sample Preparation

Samples from the Sumbawa White Honey study were taken from the Tambora Forest, Sumbawa Island, West Nusa Tenggara Province. The white honey sample is then placed in a sterile container and placed in a box at a low temperature. This is done in order to maintain and maintain the physiological condition of the sample. Furthermore, samples of Sumbawa white honey were sent to the Laboratory of Microbiology, Faculty of Medicine, University of Mataram.

White Honey Phytochemical Test

White honey samples were screened for phytochemical tests to see the content of the active compounds contained therein. Phytochemical testing includes:

Examination of Alkaloids

As much as 0.1 ml of white honey dissolved in 10 mL of 96% ethanol. The solution was then added 0.5 grams of NaCl then stirred and filtered. The filtrate obtained was then added with 3 drops of concentrated HCl and then transferred into three test tubes of 2 ml each. These three solutions were analyzed with 4-5 drops of Mayer, Dragendorff, and Wagner reagents.

Examination of Flavonoids

As much as 0.1 ml of white honey was put into a cup and added 10 mL of 96% ethanol then stirred and filtered. A total of 2 ml of the filtrate was then added with 0.1 g of Mg powder and 3 drops of 2 N HCl. Formation of yellow, orange, red or blue color indicated the presence of flavonoid compounds.

Examination of Saponins

0.1 ml of white honey is dissolved in 10 ml of water and filtered. The filtrate was put into a test tube and then shaken. Formation of stable foam with a height of 1-2 cm for 10 minutes indicates the presence of saponins.

Examination of terpenoids

As much as 1 ml of white honey solution into a test tube, chloroform and concentrated sulfuric acid were added. A reddish brown precipitate indicated the presence of terpenoids.

White Honey Dilution

The white honey dilution used in this study was at a concentration of 25%, 75% and 100%, so it is necessary to dilute the white honey sample. Here is the white honey dilution method: Take a sample of 25 mL, 75 mL, 100 mL of honey then put it into a 100 mL volumetric flask. Add the DMSO solution to the volumetric flask until the volume becomes 100 mL. Homogenize each solution.

Antibacterial testing with the Kirby Bauer method – Paper Disc Diffusion

Before carrying out an antibacterial test, the first thing that needs to be done is to carry out a bacterial suspension. Bacterial suspension was carried out by taking a colony of B. megaterium using a loop. Furthermore, the collected colonies were suspended in sterile NaCl solution. After that, make a standard McFarland 0.5 solution as a standardization of the turbidity of the NaCl suspension solution with bacteria. The results of the suspension are taken using a sterile cotton swab and squeeze the cotton swab by pressing it against the tube wall. Next, do a swab on the surface of the Muller Hinton Agar (MHA) media evenly and let it sit for 10 minutes. This is done so that the bacterial suspension seeps well into the agar media.

Calculation of the diameter of the inhibition zone

Examination or interpretation of the results of the antibacterial activity test using the Zone of Inhition Diameter (ZID) or known as the diameter of the inhibition zone which is measured using a ruler or caliper.

Result and Discussion

Phytochemicals of Sumbawa White Honey

Phytochemical screening is an initial stage in the research. The stages of phytochemical screening aim to provide an overview of the class of compounds contained in the sample, so that they can be used as drugs in curing various diseases. Phytochemical screening in this study was conducted to determine the presence of alkaloids, flavonoids, saponins and terpenoids in Sumbawa white honey.

Table 1. Phytochemical Test of Sumbawa White Honey Tubes

Test	Result	Information
Flavonoid	+	Yellow Precipitate
Alkaloid	-	No precipitae
Saponin	+	Formed foam
Terpenoid	+	Reddish brown precipitate

Figure 1. Results of phytochemical screening

Test the content of flavonoids in white honey using sinoside reagent, namely using 96% ethanol. Flavonoid compounds are the largest group of phenolic compounds. A positive result is a yellow precipitate. Based on testing white honey contains flavonoid compounds. The same result was also revealed by (ADALINA, 2017), that white honey from Sumbawa contains flavonoid compounds. Flavonoids in forest honey are derivatives of phenolic compoundsUji kandungan alkaloid pada madu putih menggunakan metode presipitasi dengan tiga perekasi yaitu Dragendorf, Wagner dan Mayer. This is presumably due to the fact that Sumba white honey has a sweet taste and does not tend to taste bitter. Most of the alkaloids taste bitter to humans (Muñoz, Schilman and Barrozo, 2020).

The results of the saponin phytochemical test showed that white honey contains saponin compounds. A positive result is indicated by the formation of foam. Saponin is a glycoside that may be present in many plants, it is possible that honey also contains saponins. It could be from bees that suck nectar from plants that contain lots of saponins. Saponin compounds are amphipathic glycosides which can foam when shaken vigorously in solution. The foam is stable and does not easily disappear (Zeru, 2021). These positive saponin results are supported by research conducted by (ADALINA, 2017).

The terpenoid test was marked by the formation of a brownish red precipitate which came from the reaction of triterpenes with acids (CH3COOH and H2SO4. Terpenoids are natural products whose structure is divided into several isoprene units, because of this this compound is also called isoprenoid (C5H8). Based on the phytochemical testing of Sumbawa white honey contains terpenoids. However (ADALINA, 2017) did not perform a terpenoid test, but did a triterpenoid test. The results were negative. Triterpenoids are derivatives of terpenoids whose carbon skeletons come from six isoprene units (2-methylbutane – 1,3 diene)

2. Antibacterial Test

Antibacterial activity in this study was tested using the disc diffusion method. The working principle of the diffusion method is the diffusion of the antibacterial compound into the solid medium where the test microbe has been inoculated. Observations were obtained in the form of the presence or absence of a clear area formed around the disc paper which indicated an inhibition zone for bacterial growth (Balouiri, Sadiki and Ibnsouda, 2016).

Based on the measurement results, it appears that variations in the concentration of Sumbawa white honey have an effect on its ability to inhibit the growth of B. megaterium bacteria. At a concentration of 100% Sumbawa white honey, the measurement results showed that the diameter of the inhibition zone was 2.59 mm. This shows that at this concentration, Sumbawa white honey has the highest antibacterial activity. Possibly, at a concentration of 100%, the antibacterial content or active substance in white honey extract starts to increase optimally so that it inhibits the growth of B. megaterium effectively.

Table 2. Measurement of the inhibition zone diameter of Sumbawa White Honey against *B. megaterium*

Treatment (Concentration)	Replication -(mm))					Average (mm)	Category
	1	2	3	4	5		
25 %	0	0	0	0	0	0	0
75 %	1.33	1.66	1.66	1.33	1.33	1.46	low
100 %	2.33	3	3	2.33	2.33	2.59	low
Negative control (DMSO)	0	0	0	0	0	0	0
Positive control (kloramfeninol)	30	30	30	30	30	30	Strong

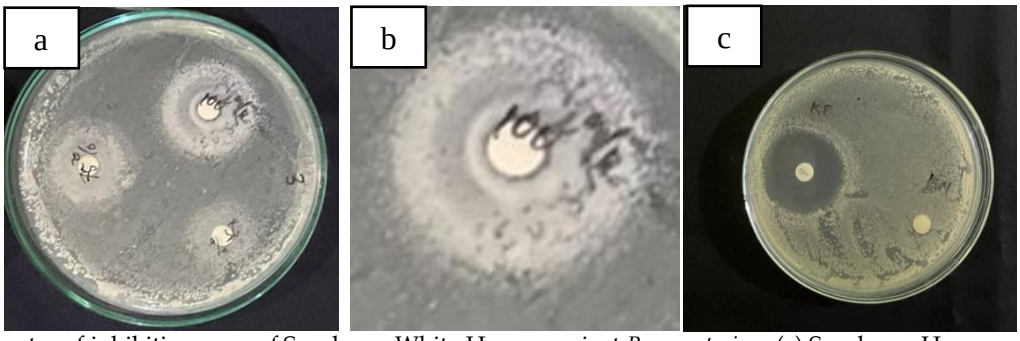

Figure 2. Diameter of inhibition zone of Sumbawa White Honey against *B. megaterium* (a) Sumbawa Honey with concentrations of 25%, 75% and 100%; (b) Concentration of 100% sumbawa honey and; (c) Positive control for chloramphenicol and negative control for DMSO

Although not as strong as 100% concentration, Sumbawa white honey at 75% concentration still has significant antibacterial activity by inhibiting the growth of B. megaterium. At a concentration of 25% Sumbawa white honey, the measurement results showed that there was no inhibition zone activity. This shows that at a concentration of 25%, the compounds contained in Sumbawa white honey are not strong enough to inhibit the growth of *B. megaterium* bacteria. However, if it is categorized as an inhibition zone, the concentration of 75% and 100% is included in the low category. This is based on the criteria for the strength of the antibacterial inhibition zone, namely the diameter of the inhibition zone of 5 mm or less is categorized as weak, the inhibition zone of 5-10 mm is categorized as medium and the inhibition zone of 10-20 mm is categorized as strong, while above 20 mm is very strong.

Mechanisms of Action of Flavonoids as Antibacterials Inhibiting Nucleic Acid Synthesis, Flavonoids, especially the A and B rings, interact with the nucleic acid bases in DNA and RNA by means of interactions or hydrogen bonds. This causes the accumulation of nucleic acid bases, inhibits the formation of DNA and RNA, and disrupts the process of genetic replication in bacterial cells. Inhibiting Cell Membrane Function is flavonoids form complex compounds with extracellular and dissolved proteins, thereby damaging the bacterial cell membrane. As a result, intracellular compounds leave the cell, disrupt the normal function of the cell, and contribute to bacterial cell death. Inhibiting Energy metabolism: Flavonoids inhibit the formation of energy in the cytoplasmic membrane of bacteria and also inhibit bacterial motility, which play a role in antimicrobial activity and extracellular proteins. Thus, flavonoids can inhibit the growth and reproduction of bacteria (Shamsudin *et al.*, 2022).

The mechanism of action of saponins as antibacterials include causing Leakage of Proteins and Enzymes from Inside the Cell. Saponins have surface properties similar to detergents, thus reducing the surface tension of the bacterial cell wall and damaging membrane permeability. This causes proteins and enzymes to move out of the cell, interferes with the survival of bacteria, and contributes to cell death. Disrupt and Reduce Cell Membrane Stability. Saponins diffuse through the outer membrane and vulnerable cell walls, then bind to the cytoplasmic membrane. This causes disruption and destabilization of the cell membrane, resulting in cytoplasm leaking out of the cell and resulting in bacterial cell death.Antimicrobial agents that disrupt the cytoplasmic membrane, such as saponins, are bactericidal, meaning they can kill bacteria.

Conclusion

Sumbawa white honey has the ability to inhibit the growth of *B. megaterium* bacteria in the low category. The higher the concentration of white honey, the higher the ability as an antibacterial. Concentration of 100% white honey is more capable of inhibiting *B. megaterium* than concentrations of 75% and 25%. The white honey sample has lower antibacterial activity, given the significant difference with the positive control. However, it is important to remember that these results only include the white honey samples studied and do not generalize to all available white honey. In addition, further research may be needed to understand the antibacterial potential of white honey in more depth. And keep in mind that these results are limited to antibacterial tests against *B. megaterium*, and further research may be needed to understand the antibacterial activity of Sumbawa white honey against various other types of bacteria.

Acknowledgments

This research activity has been carried out well, it cannot be separated from the role of several parties, therefore thanks are conveyed to: Faculty of Medicine, University Mataram, LPPM University of Mataram, Laboratory of Microbiology FK UNRAM and Laboratory of Pharmacology FK UNRAM

Author Contributions
Nurmi Hasbi: writing-original draft preparation, methodology, result, conculusion, Rosyunita: analysis, Adelia Rizka Rahim: analysis, Hagni Wardoyo: proofreading, Ida Ayu Arnawati: proffreading, Saskia Safarina Haza: editing, Lale Nandhita Hulfifa: editing, Abiel Dwi Cahya Firdaus Alamsyah: editing, I Komang Satya Validika: editing.

Funding
Antibacterial from white honey to *B. megaterium*

Conflict of Interest
The authors declare that there is no conflict of interest regarding the publication of this paper

References

Adalina., Y. (2017). Kualitas madu putih asal Provinsi Nusa Tenggara Barat. 3, pp.189–193. https://doi.org/10.13057/psnmbi/m030204

Almasaudi, S. (2021). The antibacterial activities of honey. *Saudi Journal of Biological Sciences, [online]* 28(4), 2188–2196. https://doi.org/10.1016/j.sjbs.2020.10.017

Balouiri, M., Sadiki, M. & Ibnsouda, S. (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. Epub 2015 Dec 2.

Bocchi, M.B., Perna, A., Cianni, L., Vitiello, R., Greco, T., Maccauro, G. &Perisano, C. (2020). A rare case of *Bacillus megaterium* soft tissues infection. *Acta Biomedica,* 91, 1–5. https://doi.org/10.23750/abm.v91i14-S.10849

Crisafulli, E., Aredano, I., Valzano, I., Burgazzi, B., Andrani, F.& Chetta, A. (2019). Pleuritis with pleural effusion due to a Bacillus megaterium infection. *Respirology Case Reports*, 7(1), 1–3. https://doi.org/10.1002/rcr2.381

Doron, S; Gorbach, S. (2020). Bacterial Infections : Overview. Bacteriology Amsterdam, Amsterdam, The Netherlands, 3(January). https://doi.org/10.1016/B978-012373960-5.00596-7

Duncan, K.O. and Smith, T.L. (2011). Primary cutaneous infection with Bacillus megaterium mimicking cutaneous anthrax. *Journal of the American Academy of Dermatology*, 65(2), .e60–e61. https://doi.org/10.1016/j.jaad.2011.02.024

Guo, F.P., Fan, H.W., Liu, Z.Y., Yang, Q.W., Li, Y.J. and Li, T.S. (2015). Brain abscess caused by *Bacillus megaterium* in an adult patient. *Chinese Medical Journal,* 128(11), 1552–1554. https://doi.org/10.4103/0366-6999.157697

ICON, (2023). Assessing strategies for mitigating infectious diseases.

Kwakman, P.H.S., te Velde, A.A., de Boer, L., Vandenbroucke-Grauls, C.M.J.E. and Zaat, S.A.J., (2011). Two major medicinal honeys have different mechanisms of bacteric idal activity. *PLoS ONE*, 6(3), 3–9. https://doi.org/10.1371/journal.pone.0017709

Maimunah, S., Kennedy Marpaung, J., Cinthya Eriwaty Silalahi, Y., Wahyuni Turnip, I., kunci, K., Antibakteri, A., cereus, B. and Hutan, M., (2021). Uji Aktivitas Antibakteri Madu Hutan Terhadap Bakteri Bacillus cereus Forest Honey Antibacterial Activity Against Bacillus cereus. *Jurnal Farmanesia*. 8(1), pp.9–15. https://doi.org/10.51544/jf.v8i1.2780

Manguntungi, B., Mustopa, A.Z., Meilina, L., Nurfatwa, M., Vanggy, L.R., Irawan, S., Tamzil, M.S., Aprilian, T., Yulianti, Fidduha, A.S. and Wersian, I.N., (2021). The profile analysis of lactic acid bacteria (Lab) from sumbawa white honey and its potential producing antibacterial compounds. *Walailak Journal of Science and Technology*, 18(15), pp.1–12. https://doi.org/10.48048/wjst.2021.22204

Muñoz, I.J., Schilman, P.E. and Barrozo, R.B., 2020. Impact of alkaloids in food consumption, metabolism and survival in a blood-sucking insect. *Scientific Reports*, 10(1), pp.1–10. https://doi.org/10.1038/s41598-020-65932-y

Rostinawati, T., (2009). Aktivitas Antibakteri Madu Amber dan Madu Putih Terhadap Bakteri *Pseudomonas aeruginosa* Multiresisten dan Staphylococcus aureus Resisten Metisilin. *Penelitian Mandiri*, p.1.

Shamsudin, N.F., Ahmed, Q.U., Mahmood, S., Shah, S.A.A., Khatib, A., Mukhtar, S., Alsharif, M.A., Parveen, H. and Zakaria, Z.A. (2022). Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation. *Molecules*, 27(4). https://doi.org/10.3390/molecules27041149

Zeru, M., 2021. Prevalence and associated factors of hiv-tb co-infection among hiv patients: A retrospective study. *African Health Sciences*, 21(3), pp.1003–1009. https://doi.org/10.4314/ahs.v21i3.7