ORIGINAL ARTICLE

Qualitative Phytochemical Screening and Antibacterial Effect of *Averrhoa bilimbi* Fruit Extracts Against Selected Bacteria

Nur Dayana Hassan Cheong, Faizah Saniah Ibrahim, Hartini Yusof

Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, Universiti Teknologi MARA, Selangor Branch, Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

ABSTRACT

Introduction: Most diseases involving bacterial infections have caused high mortality and morbidity rates worldwide. However, the misuse of synthetic antibiotics in treatments has not only caused adverse effects on patients but also led to the emergence of antibiotic-resistant bacteria. The escalating issue of antimicrobial resistance has led to the urgent need for new antimicrobial agents originating from natural resources. Thus, this study was conducted to determine the antibacterial activity of ethyl acetate and aqueous extracts of *Averrhoa bilimbi* fruits against selected bacteria. **Methods:** The antimicrobial susceptibility testing (AST) and minimum inhibitory concentration (MIC) by disc diffusion method and microdilution broth method were respectively performed to evaluate the antibacterial activity of the *A. bilimbi* extract against gram-positive *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 14579), as well as gram-negative bacteria (*Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 13311)). **Results:** Initial screening for ethyl acetate and aqueous extracts showed inhibition against all microbes with the most significant effects on *B. cereus* at 16 and 11.67±1.15 mm, respectively. With MIC value of 7.81 mg/ml, the lowest concentration of ethyl acetate extract was required by *S. aureus* and *B. cereus* to inhibit their growth. For aqueous extract, the lowest MIC value of 31.25 mg/ml was observed to inhibit *E. coli*. Moreover, *A. bilimbi* fruit extracts also contain alkaloids, flavonoids, phenols, terpenoids, tannins, and reducing sugars responsible for antibacterial activities. **Conclusion:** Therefore, *A. bilimbi* fruit extract with its potential antibacterial properties can be used as an alternative therapy to combat infectious diseases initiated by the selected pathogens.
Malaysian Journal of Medicine and Health Sciences (2022) 18(SUPP15): 14-20. doi:10.47836/mjmhs18.s15.3

Keywords: *Averrhoa bilimbi*, Extracts, Antibacterial, MIC, Phytochemicals

Corresponding Author:
Hartini Yusof, MSc
Email: tini01@uitm.edu.my
Tel: +603-32584431

INTRODUCTION

These days, the overuse and misuse of synthetic antibiotics in the treatment of illnesses not only has negative consequences for patients but may also contribute to the formation of bacterial resistance (1), making disease treatment increasingly difficult. Thus, studies evaluating the replacement of synthetic antibiotics with natural antimicrobial agents are in great demand.

For decades, natural resources such as fruits, leaves, stems, or barks of plants have been utilized in treating a variety of illnesses (2). According to previous research (3), plants have hundreds of bioactive chemicals that are vital to human health. Still, only a few species have had these compounds identified and used in the creation of novel pharmaceuticals. Flavonoids, phenols, triterpenoids, glycosides, and other beneficial compounds have been found in local fruits often consumed by Malaysians (4). These phytochemical compounds are potent antioxidants and offer benefits such as protection against the development of neurodegenerative diseases, cancers, vascular diseases, and diabetes (5,6). They also possess antibacterial properties that allow the fruits to be used as sources for treatment against bacterial infections (7).

Due to a seasonally humid climate, the fruits of *A. bilimbi*, also known as belimbing buluh, are readily available in Malaysia as the environment is ideal for their growth. Since the old days, these fruits have been incorporated in dishes such as curry stew and belimbing buluh chilli sauces. Scurvy, hypertension, obesity, and diabetes have all been traditionally treated with the syrup or juice of *A. bilimbi*. It has also been consumed for its ability to stop rectal bleeding (8). According to past research,

A. bilimbi fruits contain various beneficial compounds that could treat a variety of symptoms and illnesses. In recent studies, analysis of methanolic extracts of *A. bilimbi* fruit was found to possess hepatoprotective, anticancer (8), and antibacterial (9) properties. Similarly, the antibacterial properties of *A. bilimbi* fruit were also demonstrated via aqueous, chloroform, and ethanolic extractions (8). Antioxidant capacity was also shown with aqueous extraction of the fruit (10). Other than that, the ethanolic and aqueous extract of *A. bilimbi* leaves were proven to exert antifungal, antithrombotic, antidiabetic, and antihypertensive properties (8).

As bacteria become increasingly resistant to synthetic antibiotics, researchers are concentrating their efforts on natural antimicrobials, such as plant extracts to replace artificial pharmaceuticals (11,12). *A. bilimbi* fruit has been shown to have antibacterial properties in several previous research studies (10,13,14). For example, it was proven to inhibit *S. aureus*, *L. monocytogenes*, and *B. cereus*, among other gram-positive bacteria (15). Gram-negative bacteria such as *Salmonella typhi, Escherichia coli, Shigella dysenteriae*, and *Vibrio parahemolyticus* were shown to be resistant to the methanolic extract of *A. bilimbi* fruits (9). However, there are just a few studies that employed ethyl acetate as a solvent for *A. bilimbi* fruit extraction. It was found that the solubility of compounds in the plant responsible for its antibacterial properties is influenced by the solvents utilized in the extraction process (16). The knowledge of the medicinal plant could aid in reducing the country's economic losses and the health concerns associated with the development of antibiotic-resistant bacteria. Furthermore, the bioactive compounds of the *A. bilimbi* fruit can be identified and marketed for other medicinal purposes (17).

The increase in resistance in bacteria including methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci, and multidrug-resistant bacteria such as *S. typhi*, has resulted from the widespread use of synthetic antibiotics in the treatment of human diseases (18). As emerging and developing bacteria are difficult to treat and eliminate, these outcomes may raise the burden of sickness among humans. Furthermore, the overuse and abuse of synthetic antibiotics might have negative consequences for consumers (19), which include stomach aches, diarrhea, vomitings, skin rashes, and even more severe effects such as breathing difficulties and dizziness (20). Hence, replacing the usage of artificial antibiotics with natural products that are rich in bioactive compounds could aid in overcoming these effects.

This research was conducted to determine the phytochemical compounds and the antibacterial activity of the *A. bilimbi* fruit extract against selected bacteria, including *S. aureus, B. cereus, E. coli*, and *S. typhimurium*, by using ethyl acetate and aqueous solvents.

MATERIALS AND METHODS

A. bilimbi Fruits Collection and Processing

A. bilimbi fruits were collected from their natural habitat in Perak. They were washed using tap water and then cut into smaller pieces to allow for a faster drying process. With slight modification from (21), the fruits were first dried in an oven for 12 hours at 60°C to prevent fungus formation before drying under the sun for five days. Then, the dried fruits were ground into a fine powder by using a miller (22).

Preparation of *A. bilimbi* Fruit Extracts

The *A. bilimbi* fruit samples were extracted using two different solvents, ethyl acetate, and distilled water. The procedures used for both extractions were adopted from (22), with slight modification. The ratio of fruits and solvent used was 1:10. Thus, 100 gm of *A. bilimbi* fruit powder was macerated with 1 L of solvent in an orbital shaker at 150 rpm for 6 hours, followed by overnight extraction without agitation at room temperature. The mixture container was wrapped using aluminum foil (23). Next, the mixtures were filtered with Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at 50°C in the water bath with optimum reduced pressure and the ethyl acetate and aqueous extracts were obtained satisfactorily. The weights of the crude extracts obtained were then measured. For the preparation of 100% concentration of ethyl acetate and aqueous extracts, 1 gm of crude extracts was diluted in 1 ml of 10% dimethyl sulfoxide (DMSO) at a 1:1 ratio (22). The prepared extracts were stored in a refrigerator at 4°C for future use.

Antibacterial Susceptibility Testing (AST) by Disc Diffusion Method

As suggested by (22) with some modifications, 10 µl of 100% concentration of *A. bilimbi* fruit extracts for both solvents were pipetted into the sterilized filter paper discs. The discs were dried at 40°C for 10 to 15 minutes in the oven. The discs for the negative control were made using the same technique but with 10% DMSO.

Using a wire loop, 3 to 5 colonies of bacteria were inoculated into Muller Hilton (MH) broth and incubated for approximately 2 hours. 0.5 McFarland standard was used as a comparison with the turbidity of bacterial suspension to standardize the density of bacterial inoculum. A sterile cotton swab was then immersed into MH broth with bacterial suspension for 1 minute and mixed by rotating the swab several times. The excess suspension was squeezed out by gently pressing on the edge of the bijou bottle (24). Next, the bacterial suspension was uniformly smeared over Muller Hilton agar using the Kirby-Bauer method.

Using a sterile syringe, the dried discs were arranged on the surface of the cultured agar plate and pressed down gently to ensure adherence. The four different discs that

were used in each plate were ethyl acetate extracts, aqueous extracts, negative control, and positive control discs. For negative control, 10% of the DMSO disc was used, whereas the positive control was the gentamycin 30 µg disc. The distance between each disc was evenly distributed to avoid the zones of inhibition from overlapping. The AST was performed in triplicates for each organism and all cultured media plates were left in the incubator for 24 hours at 37°C. After being incubated overnight, the zones of inhibition were measured in millimeters (mm) using a ruler and recorded.

Minimum Inhibitory Concentration (MIC)

The bacterial inoculum suspension was prepared in MH broth and incubated at 37°C for a few hours, depending on how long each organism took to reach the exponential phase. With slight modifications from (25), using sterile 10% dimethyl sulfoxide, a two-fold serial dilution of 100% extract was produced to achieve the following concentrations: 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95, and 0.98 mg/ml. The diluted extracts were pipetted onto wells 1 to 11 of the microtiter plate in sequential order. 50 µl of bacterial suspension was then pipetted into well 1 to well 11 and mixed well with the existing extracts inside.

For each species, the positive, negative, and standard controls were used to confirm the validity of the data obtained. As for the negative control, a loopful of gentamycin powder was dissolved in 50 µl of DMSO before the addition of 50 µl of bacterial suspension into the well. In contrast, 50 µl of DMSO with 50 µl of bacterial suspension was added to the well for positive control. 50 µl DMSO was used as the standard control. The microtiter plate was then incubated overnight at 37°C. The MIC test was performed in duplicates for each sensitive organism in AST and the tests were labelled Set A and Set B. The lowest concentration of extracts capable of inhibiting the development of organisms that had no visible growth was determined by measuring the turbidity of the mixture in each well of an infected microtiter plate (no turbidity) (26) and recorded.

The bacteria strains that were used in this study are *S. aureus* (ATCC 25923), *B. cereus* (ATCC 14579), *E. coli* (ATCC 25922), and *S. typhimurium* (ATCC 13311). The bacteria were obtained from stock culture plates and were subcultured into nutrient agar. The bacterial stock cultures were made by dispensing 1 ml of inoculum suspension in Brain Heart Infusion (BHI) broth into 7 ml of 20% glycerol. These stock cultures of bacteria were kept at -20°C for future use.

Qualitative Phytochemical Screening of *A. bilimbi* Fruit Extracts

An aliquot (1 ml) of both ethyl acetate and aqueous extracts obtained from *A. bilimbi* fruits was subjected to qualitative phytochemical analysis to determine the presence of secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, saponins, tannins, and reducing sugars. The presence of compounds was visualized via color changes and foam production in Wagner's Test (16), Alkaline Reagent Test (27), Ferric Chloride Test (16), Salkowski Test (28), Foam Test (16), and the use of other reagents (29), according to the respective methods. The color changes and foam production were observed and recorded.

RESULTS

***A. bilimbi* Fruits Crude Extracts**

The weight and percentage of extraction from ethyl acetate and the aqueous extraction process are tabulated in Table I below. 100 gm of ground *A. bilimbi* fruits yielded 2.36% for the ethyl acetate extract and 16.2% for the aqueous extract.

Table I: Weight and percentage (w/w) of ethyl acetate and aqueous extract product of *A. bilimbi* fruits.

Extraction solvent	Weight of grounded *A. bilimbi* fruits for extraction (gm)	Weight of extraction product (gm)	Percentage of weight extraction (100%)
Ethyl acetate	100.00	2.36	2.36
Aqueous	100.00	16.20	16.20

Antibacterial Susceptibility Testing (AST)

To get more accurate and reliable results, AST of 1000 mg/ml (100%) *A. bilimbi* extracts were conducted in triplicates using the disc diffusion technique for each of the chosen organisms. The results obtained were recorded and summarized in Table II.

Minimum Inhibitory Concentration (MIC)

MIC value of extract to inhibit the growth of each organism was determined by using the micro broth dilution method. The test was performed in duplicates for accurate results. Varying strengths of inhibition were obtained for each organism with different extracts used. The results obtained were recorded and summarized in Table III.

Table II: AST result of *A. bilimbi* fruit extracts against selected pathogen.

Organism	Zone of inhibition (mm)			
	Ethyl acetate extract	Aqueous extract	Positive control	Negative control
Staphylococcus aureus (ATCC 25923)	13.33±0.58	8.17±0.76	25.33±0.58	0
Bacillus cereus (ATCC 14579)	16.00	11.67±1.15	22.67±0.58	0
Escherichia coli (ATCC 25922)	13.67±0.58	8.17±0.29	22.17±0.29	0
Salmonella typhimurium (ATCC 13311)	11.67±0.58	9.67±0.58	24.33±0.58	0

Table III: MIC result of *A. bilimbi* fruit extracts against selected pathogen tested in duplicates.

Organism	MIC (mg/ml)			
	Ethyl acetate extract		Aqueous extract	
	Set A	Set B	Set A	Set B
Staphylococcus aureus (ATCC 25923)	7.81	7.81	62.50	62.50
Bacillus cereus (ATCC 14579)	7.81	7.81	62.50	62.50
Escherichia coli (ATCC 25922)	31.25	31.25	31.25	31.25
Salmonella typhimurium (ATCC 13311)	15.63	15.63	125.00	125.00

Qualitative Phytochemical Screening of *A. bilimbi* Extracts

Phytochemicals coumpound found in ethyl acetate and aqueous extracts of *A. bilimbi* is shown in Table IV.

Table IV: Phytochemicals screening of ethyl acetate and aqueous extracts of *A. bilimbi*

Bioactive compounds	Ethyl acetate extract	Aqueous extract
Alkaloids	Negative	Positive
Flavanoids	Positive	Positive
Phenols	Positive	Positive
Terpenoids	Negative	Positive
Saponins	Negative	Negative
Tannins	Positive	Negative
Reducing Sugars	Positive	Positive

DISCUSSION

The results of this study match those observed in an earlier study done by (15) where an aqueous extract of *A. bilimbi* fruits demonstrated a higher efficacy against *B. cereus* compared to *S. aureus*. Another study done on *A. bilimbi* fruits showed the effectiveness of chloroform and methanol extracts against *S. aureus* and *B. subtilis* (13). Other bacteria showed moderate effects with the inhibition zone ranging from 11.67 - 13.67 mm for ethyl acetate and 8.17 - 9.67 mm for aqueous extract. However, similar effects on both extracts toward gram-positive bacteria, *S. aureus*, and other gram-negative bacteria were observed. This finding was slightly different from a previous study done where gram-positive bacteria yielded highly sensitive results compared to gram-negative bacteria. As gram-positive bacteria lack the outer membrane of lipopolysaccharide components which acts as a protective barrier, they are more easily penetrated by other compounds compared to gram-negative bacteria (30,31).

Overall, compared to aqueous extract, ethyl acetate extract is significantly more effective against selected bacteria due to the larger zones of inhibition observed in AST, except for *S. typhimurium* which showed the smallest zone of inhibition at 11.67±0.58 mm where the difference is not highly significant compared to 9.67±0.58 mm achieved with aqueous extract. This result was similar to the other findings where aqueous extract exhibited low antibacterial activity compared to the other extraction solvents (32,33). On the other hand, one study reported the best antibacterial activity of *A. bilimbi* water extract when tested against ESβL + CR *Pseudomonas aeruginosa* in a test against multidrug-resistant bacteria (34). This proves that the effectivity of *A. bilimbi* fruit extracts against bacteria varies according to the extraction solvents and species of bacteria tested. When MIC was conducted to test for growth inhibition, it was found that *S. aureus* and *B. cereus* required the lowest concentration of ethyl acetate extract of *A. bilimbi* fruits to inhibit their growth with MIC values of 7.81 mg/ml and 7.81 mg/ml, respectively. However, a higher MIC value of ethyl acetate extract at 15.63 mg/ml was found to be more effective to control the growth of *S. typhimurium*. On the other hand, the gram-positive bacteria showed moderate activity with a MIC value of 62.5 mg/ml for aqueous extract. The highest MIC value, 125 mg/ml was observed in inhibiting the visible growth of *S. typhimurium*. For *E. coli*, the ethyl acetate extract gave the highest MIC value, 31.25 mg/ml. But for the aqueous extract, *E. coli* required the lowest concentration of extract to inhibit it with the same MIC value as ethyl acetate extract, 31.25 mg/ml. Ethyl acetate extract of *A. bilimbi* fruits exhibited stronger antibacterial activities with lower MIC values compared to aqueous extract as supported by (35) and (36) who claimed the presence of strong antimicrobial compounds in the extract with low MIC value.

The result obtained from the MIC value of *A. bilimbi* fruit extracts could be considered high compared to the findings on the other plants as the lowest MIC value was only 7.81 mg/ml. According to (37), 0.5 and 0.1 mg/ml of an extract of *Helichrysum aureonitens* were able to inhibit *S. aureus*, while (35) found 1.25 mg/ml to be the lowest MIC value of *Zataria multiflora* extract in inhibiting selected organisms. Different phytochemical compounds present in each extract suggested an influence on the MIC values.

The alkaloids, flavonoids, phenols, terpenoids, tannins, and reducing sugars discovered in this study are responsible for the antibacterial effects (7,38). However, there was an absence of saponin in both extracts. This finding was slightly different from the previous study in (39) and (40) which showed the presence of saponin and the absence of alkaloids in the *A. bilimbi* fruit extracts. The dissimilarity between the extractive compounds present was highly probable due to the

different extraction solvents and methods used. In addition to that, a study done by (41) found the presence of alkaloids, tannins, saponins, flavonoids, cardiac glycosides, triterpenes, phenols, and carbohydrates but the absence of phytosterols in different extractives of *A. bilimbi* fruits. This is also supported by (42) which stated the presence of tannin, flavonoids, saponins, formic acid, peroxides, and triterpenoids in *A. bilimbi* fruits. Aside from fruits, the leaves of *A. bilimbi* too indicated the presence of alkaloids, tannins, and steroids (43). This shows the abundance of bioactive compounds in the species that require more attention to be utilized to their full potential.

Slightly different phytochemical compounds were detected in the ethyl acetate and aqueous extract of the *A. bilimbi* fruit. In the ethyl acetate extract, alkaloids and terpenoids were absent, while in the aqueous extract, only tannins were not present. Although more compounds were observed in the aqueous extract than in the ethyl acetate extract, it exhibited lower antibacterial properties compared to ethyl acetate extract in this study. This is probably due to the water-soluble compounds extracted by the aqueous solvent did not have any real impact on the antibacterial activity as compared to the organic solvents (44). Even so, the efficacy of *A. bilimbi* ethyl acetate extract is still commendable and is supported by a previous study (45) which showed the inhibition of *S. aureus* using the same extraction solvent. Furthermore, it was found that the juice of *A. bilimbi* which contained oxalic and ascorbic acid was ultimately able to kill bacteria in the undissociated form by penetration of the bacterial cell membrane (30).

According to (34), flavonoids, tannins, and terpenes have been proven to show significant antibacterial activities. The alkaloids, flavonoids, phenols, terpenoids, and tannins present in the extracts of *A. bilimbi* fruits exert antimicrobial action via different mechanisms (46). Alkaloids may disrupt the cell wall synthesis of the bacteria by intercalating with their DNA (44). Flavonoids bring about energy exhaustion in the bacteria by forming complexes with the bacterial proteins and suppressing their enzymes (47). According to (48), the presence of phenols interfered with the membrane permeability of the bacteria. The lipid profile of the bacterial cell wall was affected by the terpenes (49). Moreover, the appearance of tannin in bacteria's proline proteins interfered with their protein production (46). A previous study (50) suggested that the tannins are toxic to bacteria as they can cause deprivation of substrates and suppression of enzymes.

CONCLUSION

A. bilimbi fruit extracts can potentially be used as a natural antimicrobial agent to treat diseases caused by selected bacteria including *S. aureus*, *B. cereus*, *E. coli*, and *S. typhimurium*. Based on the results obtained from AST and MIC, the varying strength of antibacterial activities detected by both extracts for each type of organism does not depend on whether the organisms are gram-positive or gram-negative. In this present study, ethyl acetate extracts were found to be more effective against the selected bacteria compared to the aqueous extract. Thus, it is probably able to replace the usage of water in extracting the plant materials as a safe organic solvent that is easier to handle compared to water. The phytochemical compounds that exhibit the antibacterial properties in the extracts were alkaloids, flavonoids, phenols, terpenoids, tannins, and reducing sugars. All of these compounds have their own specific mechanisms of action against the microorganism. The presence of phytochemical compounds in *A. bilimbi* is the reason why it has been used in traditional medicine. However, in vivo studies using animal models must be conducted to assess for cytotoxic and biological side effects on humans prior to long-term usage.

More effort is needed to investigate the efficacy of the *A. bilimbi* fruits as an antibacterial agent by comparing different extraction methods or different types of solvents. In this study, the consistency of the pH of the extracts used in AST was not measured. The limited method of detection of phytochemical compounds by using the observation method to the color changes can result in observation errors. This detection method has very limited reliability because it can only be used for main compounds. Therefore, a more precise technique by using high-performance liquid chromatography (HPLC) or gas chromatography-mass spectroscopy (GC-MS) to isolate, determine, and quantify the bioactive compounds possessed by the *A. bilimbi* fruits should be considered.

ACKNOWLEDGEMENT

The authors would like to thank the Faculty of Health Sciences, UiTM Selangor Branch, Puncak Alam Campus for providing laboratory facilities and financial support.

REFERENCES

1. Mittal AK, Bhardwaj R, Mishra P, Rajput SK. Antimicrobials Misuse/Overuse: Adverse Effect, Mechanism, Challenges and Strategies to Combat Resistance. Open Biotechnol J. 2020;14(1):107–12. doi: 10.2174/1874070702014010107
2. Koehn FE, Carter GT. The evolving role of natural products in drug discovery. Nat Rev Drug Discov. 2005;4(3):206–20. doi: 10.1038/nrd1657
3. Vijayakumar A, Duraipandiyan V, Jeyaraj B, Agastian P, Raj MK, Ignacimuthu S. Phytochemical analysis and in vitro antimicrobial activity of Illicium griffithii Hook. f. & Thoms extracts. Asian Pacific J Trop Dis [Internet]. 2012;2(3):190–9. doi:10.1016/S2222-1808(12)60045-0
4. Dembitsky VM, Poovarodom S, Leontowicz H,

Leontowicz M, Vearasilp S, Trakhtenberg S, et al. The multiple nutrition properties of some exotic fruits: Biological activity and active metabolites. Food Res Int [Internet]. 2011;44(7):1671–701. doi:10.1016/j.foodres.2011.03.003
5. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009;2(5):270–8. doi:10.4161/oxim.2.5.9498
6. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines. 2018;5(3):93. doi: 10.3390/medicines5030093
7. Akter K, Barnes EC, Brophy JJ, Harrington D, Elders YC, Vemulpad SR, et al. Phytochemical Profile and Antibacterial and Antioxidant Activities of Medicinal Plants Used by Aboriginal People of New South Wales, Australia. Evidence-Based Complement Altern Med. 2016;2016. doi: 10.1155/2016/4683059
8. Saini S. A Review On Phytochemistry And Pharmacology Of Averrhoa bilimbi Linn. Int Educ Res J. 2016;2(1):71–6.
9. Das SC, Sultana S, Roy S, Hasan SS. Antibacterial and cytotoxic activities of methanolic extracts of leaf and fruit parts of the plant Averrhoa bilimbi (Oxalidaceae). Am J Sci Ind Res. 2011;2(4):531–6. doi: 10.5251/ajsir.2011.2.4.531.536
10. Noor Asna A, Noriham A. Antioxidant activity and bioactive components of oxalidaceae fruit extracts. Malaysian J Anal Sci. 2014;18(1):116–26.
11. Saeed F, Afzaal M, Tufail T, Ahmad A. Use of Natural Antimicrobial Agents: A Safe Preservation Approach. Act Antimicrob Food Packag. 2019;1–18. doi: 10.5772/intechopen.80869
12. Santos SAO, Martins C, Pereira C, Silvestre AJD, Rocha SM. Current challenges and perspectives for the use of aqueous plant extracts in the management of bacterial infections: The case-study of Salmonella enterica serovars. Int J Mol Sci. 2019;20(4). doi: 10.3390/ijms20040940
13. Abraham CM. Antibacterial Effects of Averrhoa Bilimbi L . Fruit Extracts. Int Res J Biol Sci. 2016;5(8):72–4.
14. Roy A, Rv G, Lakshmi T. Averrhoa bilimbi Linn – Nature's Drug Store- A Pharmacological Review. Int J Drug Dev Res. 2011;3(3):101–6.
15. Zakaria ZA, Zaiton H, Henie EFP, Mat jais AM, Zainuddin ENHE. In vitro antibacterial activity of Averrhoa bilimbi L. leaves and fruits extracts. Vol. 2, International Journal of Tropical Medicine. 2007. p. 96–100.
16. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A Review. Int Pharm Sci. 2011;1(1).
17. Ali Hassan SH, Fry JR, Abu Bakar MF. Phytochemicals content, antioxidant activity and acetylcholinesterase inhibition properties of indigenous *Garcinia parvifolia* fruit. Biomed Res Int. 2013;2013. doi: 10.1155/2013/138950
18. Al-zoreky NS, Al-Taher AY. Antibacterial activity of spathe from Phoenix dactylifera L. against some food-borne pathogens. Ind Crops Prod [Internet]. 2015;65:241–6. doi:10.1016/j.indcrop.2014.12.014
19. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. Perspect Medicin Chem. 2014;(6):25–64. doi: 10.4137/PMC.S14459
20. Langdon A, Crook N, Dantas G. The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. Genome Med [Internet]. 2016;8(1). doi:10.1186/s13073-016-0294-z
21. Aziz A, Rahman S, Islam M, Begu. AA. A Comparative Study on Antibacterial Activities and Cytotoxic. Int J Pharm Sci Res. 2014;5(3):913–8. doi: 10.13040/IJPSR.0975-8232.5(3).913-18
22. Yeo YL, Chia YY, Lee CH, Sow HS, Yap WS. Effectiveness of maceration periods with different extraction solvents on in-vitro antimicrobial activity from fruit of Momordica charantia L. J Appl Pharm Sci. 2014;4(10):16–23. doi: 10.7324/JAPS.2014.40104
23. Pandey A, Mishra R. Antibacterial properties of Aegle marmelos leaves , fruits and peels against various pathogens. J Pharm Biomed Sci. 2011;13(13):6.
24. Bisht R, Chanyal S, Agrawal PK. Antimicrobial and phytochemical analysis of leaf extract of medicinal fruit plants. Asian J Pharm Clin Res. 2016;9(4):131–6.
25. Nanasombat S, Lohasupthawee P. Antibacterial Activity of Crude Ethanolic Extracts and Essential Oils of Spices against Salmonella and Other Enterobacteria. KMITL Sci Technol J. 2005;5(3):527–38.
26. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 2001;48(1):5–16. doi: 10.1093/jac/dkf083
27. Hossain MA, AL-Raqmi KAS, AL-Mijizy ZH, Weli AM, Al-Riyami Q. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. Asian Pac J Trop Biomed. 2013;3(9):705–10. doi:10.1016/S2221-1691(13)60142-2
28. Alamelumangai M, Dhanalakshmi J, Mathumitha M, Renganayaki RS, Muthukumaran P, Saraswathy N. In vitro studies on phytochemical evaluation and antimicrobial activity of Borassus flabellifer Linn against some human pathogens. Asian Pac J Trop Med. 2014;7(S1):S182–5. 10.1016/S1995-7645(14)60228-5
29. Saranraj P, Stella D, Sathiyaseelan K, Samuel S. Antibacterial Potentiality of Ethanol and Ethyl Acetate Extract of Acalypha indica against Human Pathogenic Bacteria Keywords. J Ecobiotechnology

[Internet]. 2010;2(January 2010):2077–0464. Available from: http://journal-ecobiotechnology.com/

30. Wan Norhana MN, A. MNA, Poole SE, Deeth HC, Dykes GA. Effects of bilimbi (Averrhoa bilimbi L.) and tamarind (Tamarindus indica L.) juice on Listeria monocytogenes Scott A and Salmonella Typhimurium ATCC 14028 and the sensory properties of raw shrimps. Int J Food Microbiol [Internet]. 2009;136(1):88–94. doi:10.1016/j.ijfoodmicro.2009.09.011
31. Ebbensgaard A, Mordhorst H, Aarestrup FM, Hansen EB. The role of outer membrane proteins and lipopolysaccharides for the sensitivity of Escherichia coli to antimicrobial peptides. Front Microbiol. 2018;9(SEP). doi: 10.3389/fmicb.2018.02153
32. Sibi G, Naveen R, Dhananjaya K, Ravikumar KR, Mallesha H. Potential use of Muntingia calabura L . extracts against human and plant pathogens. Pharmacogn J [Internet]. 2012;4(34):44–7. doi:10.5530/pj.2012.34.8
33. Adeshina G. Antimicrobial Activity of the Aqueous and Ethyl Acetate Sub-Fractions of Alchornea cordifolia Leaf. European J Med Plants. 2012;2(1):31–41. doi: 10.9734/EJMP/2012/917
34. Prastiyanto ME, Wardoyo FA, Wilson W, Darmawati S. Antibacterial Activity of Various Extracts of Averrhoa bilimbi against Multidrug Resistant Bacteria. Biosaintifika J Biol Biol Educ. 2020;12(2):163–8. doi: 10.15294/biosaintifika.v12i2.23600
35. Saeidi S, Hassanpour K, Ghamgosha M, Heiat M, Taheri RA, Mirhosseini A, et al. Antibacterial activity of ethyl acetate and aqueous extracts of Mentha longifolia L. and hydroalcoholic extract of Zataria multiflora Boiss. plants against important human pathogens. Asian Pac J Trop Med. 2014;7(S1):S186–9. doi: 10.1016/S1995-7645(14)60229-7
36. Karon B, Ibrahim M, Mahmood A, Huq A, Chowdhury M, Hossain A, et al. Preliminary antimicrobial, cytotoxic and chemical investigations of Averrhoa bilimbi Linn. and Zizyphus mauritiana Lam. Bangladesh Pharm J. 2011;14(2):127–31.
37. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 1998;64(8):711–3. doi: 10.1055/s-2006-957563
38. Chew YL, Wan E, Chan L, Tan PL, Lim YY, Stanslas J, et al. Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia. BMC Complement Altern Med. 2011;11(12). doi: 10.1186/1472-6882-11-12
39. Huda N, Abdul B, Effendy M, Abd B, Taib MB, Bt Z, et al. Phytochemical screening and antimicrobial efficacy of extracts from Averrhoa bilimbi (Oxalidaceace) fruits against human pathogenic bacteria. Pharmacogn J. 2009;1(1):64–6.
40. Patil AG, Koli SP, Patil DA. Pharmcognostical standardization and HPTLC fingerprint of Averrhoa bilimbi (L.) fruits. J Pharm Res [Internet]. 2013;6(1):145–50. doi:10.1016/j.jopr.2012.11.030
41. Md. Hasanuzzaman MRA, Hossain M, Kuri S, Islam MS. Evaluation of total phenolic content and free radical scavenging activity of Boerhavia erecta. Int Curr Pharm J. 2013;2(4):92–6. doi: 10.1016/J.JACME.2013.06.003
42. Dewi P, Ratih GA, Burhannuddin B, Sudarmanto G. In vitro Inhibitory Activity of Ethanolic Fruit Extract from Averrhoa bilimbi L. against Streptococcus pyogenes Bacteria. Heal Notions. 2019 Jan 31;3(1):13–7. doi:10.33846/hn.v3i1.276
43. Agung S, Kusuma F, Gunawan E, Saptarini NM. Antibacterial activity of Averrhoa bilimbi leaf extract against Staphylococcus epidermidis and Staphylococcus aureus. 69 World J Pharm Res SJIF Impact Factor 8 [Internet]. 2020 [cited 2022 Apr 3];9(8):69–75. Available from: www.wjpr.net
44. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564–82. doi: 10.1128/CMR.12.4.564
45. Pertiwi D, Hafiz I, Jannah W, Sastra Winata H, Sari M, Suroyo RB. Antibacterial activities of belimbing wuluh (Averrhoa bilimbi l.) ethyl acetate extract on gel formulated against *Propionibacterium acnes* and *Staphylococcus aureus*. Artic Int J Appl Pharm [Internet]. 2020 [cited 2022 Apr 3];12(6):224–8. Available from: http://creativecommons.org/licenses/by/4.0/
46. Nallamuthu T, Viswanathan S. Phytochemical screening and antimicrobial activity of leaf extracts of Senna alexandrina Mill. against human pathogens. Int J Curr Sci [Internet]. 2012;2(January 2012):51–6. Available from: https://www.researchgate.net/publication/236646443
47. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. Sci World J. 2013;2013(December). doi: 10.1155/2013/162750
48. Heipieper HJ, Keweloh H, Rehm HJ. Influence of phenols on growth and membrane permeability of free and immobilized *Escherichia coli*. Appl Environ Microbiol. 1991;57(4):1213–7. doi: 10.1128/AEM.57.4.1213-1217.1991
49. Zwenger S, Basu C. Plant terpenoids : applications and future potentials. Acad Journals. 2008;3(February):1–7.
50. Scalbert A. Antimicrobial properties of tannins. Phytochemistry. 1991;30(12):3875–83. doi: 10.1016/0031-9422(91)83426-L