Malaysian Journal of Chemistry, 2024, Vol. 26(5), 407-416
(Special Issue: *4th IKMPB Online Symposium 2024 - Development of Chemistry to Accomplish a Sustainable World*)

Oxalic Acid from *Averrhoa bilimbi* L. as a Bleaching Agent

Amira Dayana Hazmi, Azza Faiza Mohammad Taib, Solhan Yahya and Nurul Zawani Alias*
Fakulti Sains Gunaan, Universiti Teknologi MARA Cawangan Perlis, Kampus Arau,
02600 Arau, Perlis, Malaysia
*Corresponding author (e-mail: zawani299@uitm.edu.my)

Commercial products like Clorox have been used widely to remove stains. However, concerns have arisen regarding their environmental impact and toxicity. Therefore, oxalic acid, a bleaching agent from *Averrhoa bilimbi* fruit and leaf extract was used as an alternative to remove stains. This study determined the concentration of oxalic acid in *Averrhoa bilimbi* fruits and leaves using HPLC-UV spectroscopy. This study also aimed to formulate *A. bilimbi* fruit and leaf extracts as bleaching stain removers. Additionally, the extracts' effectiveness as stain removers was evaluated through a visibility test and compared to a commercialized stain remover. The concentrations of oxalic acid in fruit and leaf are 294.20±47.35 and 239.81±60.50 ppm, respectively. The results show that the preferred formulations are F3 (50% *A. bilimbi* fruit extract) and L3 (50% *A. bilimbi* leaf extract). Hence, a 50% solution of fruit or leaf extract can remove various types of stains, like pencil lead, tomato sauce, eye makeup, and tea, in 10 minutes. The pH test also shows that the pH of these formulations is lower than a commercialized stain remover at 10.90 for F3 and 10.91 for L3, while the pH for the commercialized stain remover is 11.86. Hence, it is proven that both fruit and leaf extracts of *A. bilimbi* can be used as an alternative stain remover.

Keywords: *Averrhoa bilimbi*; oxalic acid; stain remover; sodium hypochlorite

Received: January 2024; Accepted: July 2024

Averrhoa bilimbi, also known as belimbing buluh, is a fruit with antibacterial, astringent, antiscorbutic, and anti-inflammatory properties [1-3]. It is popular in Asian countries like Malaysia, Indonesia, and Thailand. It is also widely used in cooking around Malaysia's east coast states of Pahang, Kelantan, and Terengganu to produce 'sambal hitam'. Damayanti *et al*. (2021) reported the presence of saponins, tannins, steroids, flavonoids, and alkaloids in the ethanol extract of *A. bilimbi* leaves [4]. Other than that, the fruit juice has high levels of oxalic acid and therefore, may be used to remove iron-rust stains from clothes and to impart shine to brassware [5]. Aldesimo *et al.* (2017) reported that oxalic acid in pineapple peel extract with the addition of sodium chloride (salt) is effective against mud (protein stain), rust (stain requires special treatment methods), and deodorant (stain requires special treatment methods) [6]. Meanwhile, Hitalia *et al.* (2012) reported that oxalic acid in *A. bilimbi* fruits is effective in removing soy sauce (tannin stain) on the fabrics [7].

Oxalic acid is known as a weak acid. According to Manzak and Inal (2014), oxalic acid is used in industry as a bleaching agent, a radiator cleaner, in radiography, and as a rust remover [8]. Besides, it is approved as a disinfectant to inhibit bacteria and germs and as an inert component in pesticide composition. Additionally, it can address the environmental concern associated with sodium hypochlorite, which degrades rapidly in both anaerobic and aerobic conditions. At room temperature, oxalic acid does not volatilize or concentrate in aquatic organisms, and it degrades rapidly in surface waters and soil surfaces. It breaks down into carbon dioxide and water [9]. Oxalic acid is the compound with highest acidity found in the *Oxalidaceae* family. According to Chiang (2020), the ripe fruit of *A. bilimbi* contains about 8.45 to 10.8 mg/g of oxalic acid while its green fruit contains about 10.5 to 14.7 mg/g of oxalic acid [10]. Meanwhile, the ripe and green fruits of *A. carambola* contain 9.6 mg/g and 5.0 mg/g of oxalic acid, respectively [11, 12]. Previous studies only evaluated the concentration of oxalic acid in *A. bilimbi* and *A. carambola* fruits but not their leaves.

A stain is a mark or spot that may occasionally be seen on the surface of any substance or element. It is classified into four types: enzymatic, oxidizable, greasy, and particle. Bleaching agents are chemicals that can be used to remove stains. Through oxidation reactions, the dirt color will fade. Some of the solvents that can be used to remove stains include oxidizing agents (hydrogen peroxide), reducing solvents (sodium hydrosulfite, sodium hypochlorite, and sodium chloride), lacquer solvents, inert solvents, and detergents. All these are examples of oxidators that can be used to remove stains [13]. Thus, these oxidators are usually used to produce commercialized stain removers. According to Seiphetlheng (2015), sodium hypochlorite – derived from a mixture of sodium hydroxide and chlorine gas – is the active ingredient in commercialized stain removers [14]. However, the use of sodium

†Paper presented at the 4th IKMPB Online Symposium 2024 – Development of Chemistry to Accomplish a Sustainable World

hypochlorite has created environmental concerns, in addition to handling and storage safety issues [15]. Therefore, this study aims to determine the concentration of oxalic acid in *A. bilimbi* fruit and leaf extracts by using HPLC-UV spectroscopy. This study also aims to formulate a bleaching stain remover from *A. bilimbi* fruit and leaf extracts and their effectiveness in removing stains will be compared with commercial stain removers.

EXPERIMENTAL

Chemicals and Materials

Chemicals

Several chemicals used in this study such as methanol, standard oxalic acid, and sodium hypochlorite were purchased from Sigma-Aldrich. All the chemicals were obtained from the laboratory of UiTM Perlis Branch, Arau Campus while the standard oxalic acid was purchased from Dulab.

Materials

The samples of *Averrhoa bilimbi* L. (belimbing buluh) fruits and leaves were purchased from Ecopro Training Centre (ETC), Kg Nesam, Perlis. The plant specimen was submitted to the Herbarium Universiti Kebangsaan Malaysia for identification. The voucher number assigned to the species is ID109/2024.

Methodology

1. Preparation of Fruit and Leaf Extracts

The fresh leaves and fruits of *Averrhoa bilimbi* were cleaned with tap water. Then, they were dried at 65 °C in an oven until a constant mass was gained. After that, they were ground to a fine powder and 40 g of this powder was weighed into a 500 mL Erlenmeyer flask. Later, 400 mL of methanol was added into the flask to submerge the sample, which was then macerated for 3 days. After 3 days, the mixture was filtrated, weighed, and dried at 45 °C by a rotary evaporator. The dry crude extract was stored in an air-tight container and kept in a refrigerator for further analysis [16]. The extracts solution of 1000 ppm was prepared by dissolving 10 µg of extract with distilled water in a 10 mL volumetric flask, as shown in the subsequent equation:

$$1000\ ppm = \frac{10\ \mu g\ of\ sample/standard}{10\ mL\ of\ solvent}$$

2. Preparation of Standard Solution

The standard solution of 1000 ppm was prepared by dissolving 10 µg of oxalic acid with distilled water in a 10 mL volumetric flask. Then, distilled water was added until it reached the calibration mark. The solution was then subjected to dilution into a series of standard solutions of 500, 400, 300, 200, and 100 µg/mL [17-19].

3. High Liquid Performance Chromatography-Ultraviolet (HPLC-UV) Analysis

The quantification of oxalic acid in the extracts was carried out on Perkin Elmer series 200 HPLC consisting of a pump, autosampler, UV/Vis detector, column oven, and solvent tray. *Averrhoa bilimbi* extracts (1000 ppm) were evaluated using a C18 column in this study. The instruments' flow rate was set at 1.0 mL/min using acetonitrile: water (70:30) as the mobile phase component. Approximately 10 µL of the sample was then injected using a syringe. The wavelength had been set at 210 nm. Next, the retention period was determined by injecting 10 µL of standard oxalic acid [18, 19]. The standard calibration curve was plotted using area (µV.s) vs. concentration (ppm). The concentration of oxalic acid in fruit and leaf extracts was calculated using this calibration curve [19].

4. Formulation of Bleaching Stain Remover

Clorox was used as the standard to be compared with other formulations. The stain remover was formulated using different concentrations of *A. bilimbi* fruit and leaf extracts i.e. 100% (v/v) (F1/L1), 75% (v/v) (F2/L2), 50% (v/v) (F3/L3), and 25% (v/v) (F4/L4). The fruit or leaf extract concentration was 1000 ppm [11]. Table 1 shows the formulation of stain remover.

Table 1. Formulation of stain remover.

Formulation	**Sodium hypochlorite (mL)**	***Averrhoa bilimbi* fruits or leaves (mL)**
F1/L1 (100% v/v)	0	50.00
F2/L2 (75% v/v)	12.50	37.50
F3/L3 (50% v/v)	25.00	25.00
F4/L4 (25% v/v)	37.50	12.50
Standard (Clorox)	0	0
Control	50.00	0

5. Stain Remover Analysis

pH Test

The pH value of the formulation was determined using a pH meter. The instrument was calibrated first with a standard neutral pH buffer solution (pH 7.01) and an acid buffer solution (pH 4.01) until the pH value was displayed. The electrode was then rinsed with distilled water and dried with a tissue [20].

Staining

A sheet of cotton fabric was purchased from a nearby store in Arau, Perlis. The fabric was cut into 8 × 9 cm pieces. Then, 2 mL of stains i.e. mud, eye makeup, pencil lead, tomato sauce, and tea were spread on the fabric pieces, and these stained fabric pieces were set aside for at least 10 hours. After 10 hours, the fabric pieces were treated with different types of stain remover formulations for 10 min at 200 rpm using a magnetic stirrer before being rinsed with water [6].

Stain Visibility

For stain visibility, five individuals evaluated and determined the stains on the fabric pieces based on AATCC Test Method 130 – the standard for stain-release replica. The effectiveness of the formulations was compared by the visibility of stains on the fabric as evaluated by the five individuals. The ratings were recorded as in Table 2 and Figure 1 [21]. Table 2 shows the stain release rating, while Figure 1 shows the stain release replica.

Statistical Analysis

All data were plotted and calculated using MS Excel (Microsoft Excel 2021). The determination of oxalic acid concentration was performed in triplicate, and results were expressed as mean ± standard deviation. The significance of the difference between the two samples was tested using a one-way ANOVA test. P-values < 0.05 were considered significant for the study [22].

RESULTS AND DISCUSSION

Maceration Analysis

In this study, the maceration extraction technique was used as it is a cost-effective and straightforward method. However, it is time-consuming and takes several days to complete [23]. The process involves immersing the sample in a solvent, such as methanol, ethanol, ethyl acetate, or distilled water for a few days [24]. In this study, methanol was used as the solvent due to its high yield percentage [25].

The extraction yield of the crude extract is a crucial parameter to assess the effectiveness of the extraction process. The amount of a desired compound retrieved from plant material can be referred to as the extraction yield of a crude extract. It is a significant consideration when evaluating the quality of a crude extract since it can alter the potency and efficacy of the final product. Obtaining a higher extraction yield can simplify the process of standardizing an extract's concentration. This is particularly valuable for conducting clinical trials or other studies.

Table 2. Stain release rating [21].

Rating	Description
1	Residual stain equivalent to Replica 1
2	Residual stain equivalent to Replica 2
3	Residual stain equivalent to Replica 3
4	Residual stain equivalent to Replica 4
5	No residue stain

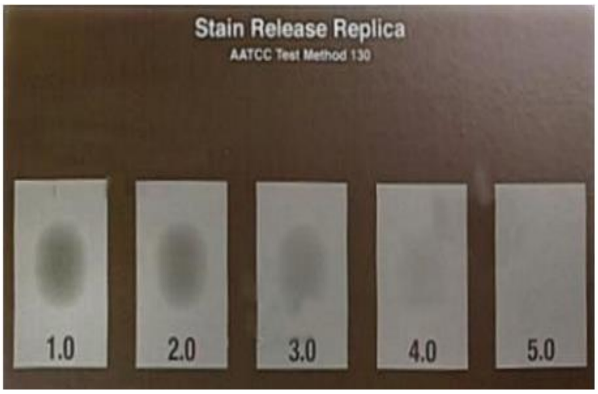


Figure 1. Stain release replica [21].

$$The\ percentage\ yield\ of\ extract\ \%(w/w) = \frac{mass\ of\ crude\ extract}{mass\ of\ sample} \times 100\%$$

Additionally, a larger extraction yield could facilitate the production of extracts with greater consistency, which is crucial for commercial applications [26]. The percentage yield of *A. bilimbi* fruit and leaf extracts was calculated using the equation shown below [27].

The percentage yields for fruit and leaf extracts were 36.93 and 14.90% (w/w), respectively. The fruits have a higher percentage yield due to their function as reproductive organs of plants. Besides, they are usually harvested when they are ripe. Hence, they are at their most nutritious and flavorful due to their high-water content [28, 29].

HPLC-UV Analysis

HPLC analysis was conducted to determine the concentration of oxalic acid in fruit and leaf extracts of *Averrhoa bilimbi*. Reverse-phase chromatography with a C18 column was used in the HPLC analysis because oxalic acid is a polar compound. Acetonitrile and water were used as the mobile phase composition to achieve optimal separation. According to the previous study by Sharma and Devi (2018), methanol and water were used as the mobile phase of HPLC analysis for oxalic acid [18]. However, methanol is not preferable as it causes high column back pressure and may lead to column failure [30]. Besides, acetonitrile exhibits lower UV absorbance than methanol [31]. Hence, it is compatible with oxalic acid as the optimal detection wavelength is 210 nm [17].

The peak area or peak height obtained from the chromatograms can be correlated with the corresponding concentrations to construct a calibration curve. This curve can then be used to determine the concentration of oxalic acid in the *Averrhoa bilimbi* extract. The calibration curve of oxalic acid standard exhibits a linear relationship between the concentration and area of oxalic acid. The determination coefficient R^2 is 0.9637 indicating that good data fit the regression line, proving the accuracy of the calibration. The linear equation of this calibration is y = 173.36x + 56091. The concentration of oxalic acid in *A. bilimbi* fruit and leaf extracts was calculated using response factor (Rf) as shown below:

$$Rf = \frac{area\ of\ standard}{concentration\ of\ standard}$$

$$concentration\ of\ sample = \frac{1}{Rf} \times area\ of\ sample$$

The Rf value obtained is 297.705. The concentration of *A. bilimbi* fruit and leaf extracts was calculated and tabulated in Table 3. The concentration of oxalic acid for both samples was not significant since P-value > 0.05. From the calculation, the concentration of oxalic acid obtained from the *A.bilimbi* fruits was higher than those from leaves at 294.20±47.35 ppm and 239.81±60.50 ppm, respectively. Hence, the result is consistent with Lúcia *et al.* (2001) and Sá *et al.* (2019) which reported that the oxalic acid in ripe fruits is 8.57 ppm while the leaf has 5.45 ppm oxalic acid [5, 32]. However, it is vital to remember that oxalic acid concentrations might change depending on a variety of factors such as genetics, soil, location, season, and maturity stage [5]. These factors can affect the overall concentration of oxalic acid in *Averrhoa bilimbi* fruits and leaves.

Stain Remover Analysis

pH Test

The pH test is important while formulating a bleaching solution to determine how basic or acidic a solution is. An acidic solution will provide a pH of less than 7 while a basic solution will provide a pH of more than 7. According to the data presented in Table 4.2, the pH of sodium hypochlorite is 12.6. This is in line with a study by Christensen *et al.* (2008), which stated that sodium hypochlorite from the manufacturer typically has a pH of 12 [33]. In this study, the pH of *Averrhoa bilimbi* fruits observed is 4.38, while the pH for leaf extract is 6.34. This is supported by Orwa *et al*. (2009), which reported that the pH of *A. bilimbi* fruits is 4.47 [34]. The pH for all formulations with sodium hypochlorite (F2, F3, F4, L2, L3, and L4) ranges from 10.63 to 11.05. There is no significant difference in the pH reading for all the formulations that had been combined with sodium hypochlorite. We can see that pH increases with the increased concentration of sodium hypochlorite. This increase is because when an alkali is added to an acidic solution, the alkali's hydroxide ions (OH-) combine with the acid's H^+ ions to generate water. This reaction lowers the concentration of H^+ ions in the solution, raising the pH [35].

The pH value of the solution must be compatible with the skin's pH, which ranges between 4.5 and 6.5 [36]. This is because human skin shields the body from external stimuli, including chemical disturbances. However, only pure extracts of fruits and leaves, which are F1 and L1, are suitable for use directly on the skin because their pH levels are 4.38 and 6.34, respectively. In contrast, other formulations in this study do not match the skin's pH range because their pH is between 10 and 11. Hence, it is advisable to take extra care while handling these formulations by wearing a glove. Table 4 shows the pH values of the formulated stain remover.

Table 3. Concentration of oxalic acid in *Averrhoa bilimbi* fruit and leaf extracts.

Sample	Mean area (µV.s)	Concentration of oxalic acid (ppm)
Averrhoa bilimbi fruits	87582.55	294.20±47.35**
Averrhoa bilimbi leaves	71392.58	239.81±60.50**

Note: Values of concentration of oxalic acid are means of triplicate measurements, mean ± SD, and expressed in ppm. The P-value was determined using a one-way ANOVA test.
**Not significant ($p > 0.05$)

Table 4. pH value of formulated stain remover.

Formulation	pH Value
Standard (Clorox)	11.86
Control (Sodium hypochlorite)	12.06
Fruit extract 100% (F1)	4.38
Fruit extract 75% (F2)	10.63
Fruit extract 50% (F3)	10.90
Fruit extract 25% (F4)	11.04
Leaf extract 100% (L1)	6.34
Leaf extract 75% (L2)	10.66
Leaf extract 50% (L3)	10.91
Leaf extract 25% (L4)	11.05

Stain Visibility

The stain visibility test was carried out by five volunteers. Table 5 shows the average stain visibility rating as rated by these volunteers. The visibility test was carried out under consistent and natural lighting conditions to avoid color perception differences. All five volunteers also viewed the stains from the same distance and angle to maintain consistency in perception. Based on the data compiled in Tables 5, 6 and 7, the preferable formulations for removing fabric stains are F3 and L3. Despite no significant pH difference between the formulations, F3 and L3 formulations managed to remove four out of five stains within 10 minutes (tomato sauce, pencil lead, eye makeup and tea stains) compared to other formulations. This is because a specific ratio of surfactant in F3 and L3 could be more efficient at breaking down an emulsifying stain even with the small difference in pH. F3 and L3 formulations contain a combination of sodium hypochlorite and extract with ratios of 1:1. Hence, the results are in line with Bimbao *et al.* (2017) who reported a 50% concentration of *Averrhoa carambola* as being most effective to remove stains [11]. Table 5 also shows that F3 and L3 formulations are better than the commercialized stain remover which could only remove two kinds of stains, tomato sauce and tea, within 10 minutes. These results are supported by Aldesimo *et al*. (2017) and Hitalia *et al*. (2021) who mentioned that oxalic acid managed to remove pencil lead and tea stains [6, 7]. Hence, the formulations containing 50% oxalic acid (F3 and L3) can be an alternative stain remover due to their effectiveness.

Table 5. Average stain visibility rating of formulations after 10 min stirring at 200 rpm using a magnetic stirrer by five volunteers.

Stain	Formulation									
	Std	**Ctrl**	**F1**	**F2**	**F3**	**F4**	**L1**	**L2**	**L3**	**L4**
Mud	3	3	3	3	3	3	3	3	3	3
Tomato sauce	5	5	5	5	5	5	4	5	5	5
Pencil lead	4	4	3	4	5	4	3	4	5	4
Eye makeup	4	4	3	5	5	4	3	5	5	4
Tea	5	5	4	5	5	5	4	5	5	5

Note: (Std) Standard, (Ctrl) Control, (F1) 100% Fruit Extract, (F2) 75% Fruit Formulation, (F3) 50% Fruit Formulation, (F4) 25% Fruit Formulation, (L1) 100% Leaf Extract 100%, (L2) 75% Leaf Formulation, (L3) 50% Leaf Formulation, (L4) 25% Leaf Formulation, (1) Residual equivalent to Replica 1, (2) Residual equivalent to Replica 2, (3) Residual equivalent to Replica 3, (4) Residual equivalent to Replica 4, (5) No stain residue.

Table 6. Stain visibility using F3 after 10 min stirring at 200 rpm using a magnetic stirrer.

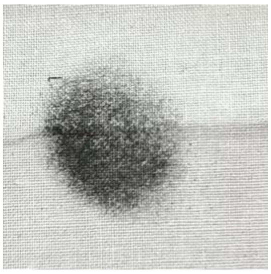
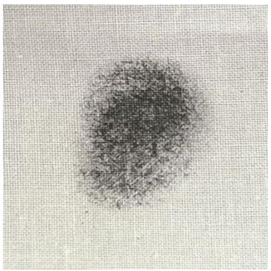
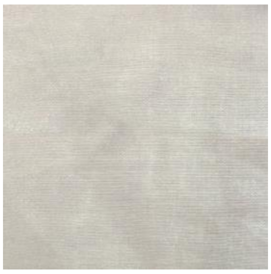
Type of stain	Before	After 10 min
Mud		
Tomato sauce		
Pencil lead		
Eye makeup		
Tea		

Table 7. Stain visibility using L3 after 10 min stirring at 200 rpm using a magnetic stirrer.

Type of stain	Before	After 10 min
Mud		
Tomato sauce		
Pencil lead		
Eye makeup		
Tea		

In F3 and L3 formulations, 50% sodium hypochlorite was used as it plays a dominant role due to its strong oxidative properties while a pure extraction sample could not efficiently remove a variety of stains. Besides, NaOCl is highly reactive and works quickly, which can make it a dominant agent in processes requiring rapid and thorough bleaching. In addition to being a bleaching agent, oxalic acid is a chelating agent, which means it may attach to metal ions and prevent them from interacting with other molecules [37]. The equation below shows the chemical reaction of oxalic acid in removing rust stain (1) [38] and sodium hypochlorite in removing stains through oxidation (2) [13].

$$Fe_2O_3.H_2O + 6H_2C_2O_4 \rightarrow 2FeC_2O_4.3H_2O + 6H_2O \quad (1)$$

$$OCl^- + H_2O + 2e^- \rightarrow Cl^- + 2OH^- \quad (2)$$

Oxalic acid is good for removing stains that are caused by metallic ions such those in tomato sauce [39], eye makeup, and tea [40]. According to Aldesimo *et al.* (2017), oxalic acid in pineapple peel manages to remove mud [6]; however, this result is in contrast with this study. In this study, mud could not be removed by all the formulations. While mud does contain metallic ions as part of its mineral content, the presence and concentration of these ions can vary widely depending on the source of the mud. The effectiveness of cleaning formulations on mud stains may not be solely dependent on the presence of metallic ions but also on the overall composition of the mud and the nature of the stain [41].

CONCLUSION

In conclusion, *Averrhoa bilimbi* fruits and leaves were successfully extracted by maceration with a percentage yield of 36.93 and 14.90% (w/w), respectively. HPLC analysis showed that the fruits contain higher oxalic acid concentrations than the leaves at 294.20±47.35 and 239.81±60.50 ppm, respectively. Stain remover analysis was successfully conducted, and the results show that the preferable formulations are F3 and L3, which contain a ratio of 1:1 fruit or leaf extract and sodium hypochlorite due to both formulations managing to remove four out of five stains. The stains removed were tomato sauce, pencil lead, eye makeup, and tea. pH test showed that the pH of commercialized stain remover is higher than the pH for F3 and L3. Hence, the formulations with the addition of oxalic acid can be used as an alternative to remove stains, but extra caution is needed due to the high pH of both formulations.

ACKNOWLEDGEMENTS

The authors would like to thank the Universiti Teknologi MARA for research grant 600-RMC/ LESTARI SDG-T 5/3 (106/2019).

REFERENCES

1. Dewi, P., Ratih, G. A. & Sudarmanto, G. (2019) In vitro inhibitory activity of ethanolic fruit extract from *Averrhoa bilimbi* L. against streptococcus pyogenes Bacteria. *Health Notions*, **3(1)**, 13–17.

2. Pérez-Calderón, J., Santos, M. V. & Zaritzky, N. (2020) Synthesis, characterization and application of cross-linked chitosan/oxalic acid hydrogels to improve azo dye (Reactive Red 195) adsorption. *Reactive and Functional Polymers*, **155**, 104699.

3. Saini, S. (2016) A Review on phytochemistry and pharmacology of *Averrhoa bilimbi* Linn. *International Education and Research Journal*, **2(1)**, 71–76.

4. Damayanti, I. A. M., Indrayoni, P., Antari, N. W. S. & Padmiswari, A. A. I. M. (2021) Effectiveness of *Averrhoa bilimbi* leaf extract on spermatogenic cells of mice (Mus Musculus L.) hyperglycemia. *International Journal of Health & Medical Sciences*, **4(2)**, 273–279.

5. Lima, V. L. A. G. D., Mélo, E. D. A. & Santos Lima, L. D. (2001) Physicochemical characteristics of bilimbi (*Averrhoa bilimbi* L.). *Revista Brasileira De Fruticultura*, **23(2)**, 421–423.

6. Aldesimo, A. M. O., Cabantog, W. B. & Ansay, M. R. C. (2017) Use of pineapple peels as organic stain removal and whitening agent on fabrics. *Technical Education and Skills Development Authority*. *Taguig City: TESDA Women's Center*.

7. Hitalia, J. P., Bringuelo, Y. J., Jordan, I. H., Martinez, E., Jr, R. L., Vadez, A. G., Abusama, H. & Paculanan, A. (2021) Kamias (*Averrhoa bilimbi*), Starfruit (*Averrhoa carambola*), and Karamay (*Phyllanthus acidus*) fruit extract as alternative stain remover. *ASEAN Journal of Science and Engineering*, **1(1),** 19–22.

8. Manzak, A. & Inal, M. (2014) Investigation of the oxalic acid extraction with different extractant in the emulsion type liquid membrane. In *The 2014 World Congress on Advances in Civil, Enviromental, and Material Research (ACEM14).* Edited by Chang-Koon Choi. Busan, Korea: ACEM14.

9. Schuler, E., Demetriou, M., Shiju, N. R. & Gruter, G. -J. M. (2021) Towards sustainable oxalic acid from CO2 and biomass. In *ChemSusChem*, **14(18)**, 3636–3664.

10. Chiang, M. (2020) Is excessive intake of kamias fruit (*Averrhoa bilimbi*) bad for your health? A case report and review. *International Journal of Clinical Case Reports and Reviews*, **3(5)**, 1–2.

11. Bimbao, E. G. A., Bulaclac, K. J. C., Pesasico, P. M., Superar, A. C. & Tuma-ob, J. S. L. (2017) The production of alternative bleaching stain remover from star fruit (*Averrhoa carambola*) extract. *Western Institute of Technology*.

12. Love, K. & Paull, R. E. (2011) Bilimbi. In *Fruits and Nuts, Honolulu (HI): University of Hawaii*, 1-6.

13. Zubir, M., Darmana, A., Damanik, M., Indriati Nasution, H., Annauli, O. G., Coirala Rama Uli Siregar, V. & Santika Silitonga, Y. (2020) Bleach effectively in removes the stubborn stains. *Indonesian Journal of Chemical Science and Technology,* **3(1)**, 20–24.

14. Seiphetlheng, K., Steyn, H. J. H. & Schall, R. (2017) Anolyte as an Alternative Bleach for

Cotton Fabrics, *Journal of Consumer Sciences*, **2**, 12–23.

15. Chung, I., Ryu, H., Yoon, S. Y. & Ha, J. C. (2022) Health effects of sodium hypochlorite: review of published case reports. In *Environmental Health and Toxicology*, **37(1)**, e2022006-0.

16. Iwansyah, A. C., Desnilasari, D., Agustina, W., Pramesti, D., Indriati, A., Mayasti, N. K. I., Andriana, Y. & Kormin, F. B. (2021) Evaluation on the physicochemical properties and mineral contents of *Averrhoa bilimbi* L. leaves dried extract and its antioxidant and antibacterial capacities. *Food Science and Technology*, **41(4)**, 987–992.

17. Yu, C., Wang, Y., Cao, H., Zhao, Y., Li, Z., Wang, H., Chen, M. & Tang, Q. (2020) Simultaneous determination of 13 organic acids in liquid culture media of edible fungi using high-performance liquid chromatography. *BioMed Research International*, **2020**, 2817979.

18. Sharma, P. B. & Devi, H. S. (2018) Optimization of an HPLC method for the simultaneous quantification of the major organic acids in different fruit extracts. *Journal of Pharmacognosy and Phytochemistry*, **7(6)**, 611–616.

19. Ergönül, P. G. & Nergiz, C. (2010) Determination of organic acids in olive fruit by HPLC. *Czech J. Food Sci*, **28(3),** 202–205.

20. Nurussakinah, Marbun, N., Fhitriana, S., Leny, L. & Daeli, Y. (2022) Preparation and evaluation of moisturizing sheet mask of lady finger (*Abelmoschus esculentus* L.) extract. *Journal of Drug Delivery and Therapeutics*, **12(3)**, 14–19.

21. Musa, N. S., Abdurahman, N. L., Zainal Abidin, Z., Adnan, F. H. & Nasir, E. (2021) Comparison between homemade stain remover and commercial stain remover for textiles. *Scientific Research Journal*, **18(1)**, 83.

22. Kebede, T., Gadisa, E. & Tufa, A. (2021) Anti-microbial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. *PLoS ONE*, **16(3)**, e0249253.

23. Naviglio, D., Scarano, P., Ciaravolo, M. & Gallo, M. (2019) Rapid Solid-Liquid Dynamic Extraction (RSLDE): A powerful and greener alternative to the latest solid-liquid extraction techniques. *Foods*, **8(7)**, 245.

24. Patel, K., Panchal, N. & Ingle, Dr. P. (2019) Techniques adopted for extraction of natural products extraction methods: maceration, percolation, soxhlet extraction, turbo distillation, supercritical fluid extraction. *International Journal of Advanced Research in Chemical Science*, **6(4)**, 1–12.

25. Truong, D. -H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H. & Nguyen, H. C. (2019) Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and *in vitro* anti-inflammatory activities of *Severinia buxifolia*. *Journal of Food Quality*, **2019(1)**, 8178294.

26. Zhang, Q. -W., Lin, L. -G. & Ye, W. -C. (2018) Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Medicine*, **13(1)**, 20.

27. Manandhar, S., Luitel, S. & Dahal, R. K. (2019) In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria. *Journal of Tropical Medicine*, **2019(1)**, 1895340.

28. Brazel, A. J. & Ó'Maoiléidigh, D. S. (2019) Photosynthetic activity of reproductive organs. *Journal of Experimental Botany*, **70(6)**, 1737–1754.

29. Staszowska-Karkut, M. & Materska, M. (2020) Phenolic composition, mineral content, and beneficial bioactivities of leaf extracts from black currant (*Ribes nigrum* L.), Raspberry (*Rubus idaeus*), and Aronia (*Aronia melanocarpa*). *Nutrients*, **12(2)**, 463.

30. Pearson, James D. (2004) HPLC Separation of Peptides. In William J. Lennarz and M. Daniel Lane (eds.), *Encyclopedia of Biological Chemistry*. *Elsevier: New York*, 398–403.

31. Desai, A. M., Andreae, M., Mullen, D. G., Banaszak Holl, M. M. & Baker, J. R. (2011) Acetonitrile shortage: use of isopropanol as an alternative elution system for ultra/high performance liquid chromatography. *Analytical Methods*, **3(1)**, 56–58.

32. Sá, R. D., Vasconcelos, A. L., Santos, A. V., Padilha, R. J. R., Alves, L. C., Soares, L. A. L. & Randau, K. P. (2019) Anatomy, histochemistry and oxalic acid content of the leaflets of *Averrhoa bilimbi* and *Averrhoa carambola*. *Revista Brasileira de Farmacognosia*, **29(1)**, 11–16.

33. Christensen, C. E., McNeal, S. F. & Eleazer, P. (2008) Effect of lowering the pH of sodium hypochlorite on dissolving tissue in vitro. *Journal of Endodontics*, **34(4)**, 449–452.

34. Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. & Simons, A. J. (2009) Agroforestree database : a

tree reference and selection guide, version 4.0. In *World Agroforestry Centre*.

35. Shabalala, A. N., Ekolu, S. O., Diop, S. & Solomon, F. (2017) Pervious concrete reactive barrier for removal of heavy metals from acid mine drainage − column study. *Journal of Hazardous Materials*, **323**, 641–653.

36. Ningsih, D. R., Zusfahair, Z., Kartika, D. & Fatoni, A. (2017) Formulation of hand sanitizer with antibacterials substance from n-hexane extract of oursop leaves (*Annona muricata* Linn). *Malaysian Journal of Fundamental and Applied Sciences*, **13(1)**, 1–5.

37. Nakamura, T., Tsukizawa, T. & Oya, M. (2022) Combined use of reducing agents and bio-degradable chelating agent for iron rust removal. *Journal of Oleo Science*, **71(4)**, 493–504.

38. Zumdahl, S. S. & Zumdahl S. A. (2013) Chemistry, 9th edition. Brooks/Cole.

39. Muniz, L. P., Santos, L. M. G. D., Couto, K. L. M. D. & Jacob, S. D. C. (2018) Evaluation of metals in tomato sauces stored in different types of packaging. *Food Science and Technology*, **38(3)**, 383–389.

40. Zhang, J., Yang, R., Chen, R., Peng, Y., Wen, X. & Gao, L. (2018) Accumulation of Heavy Metals in Tea Leaves and Potential Health Risk Assessment: A Case Study from Puan County, Guizhou Province, China. *International Journal of Environmental Research and Public Health*, **15(1)**.

41. Alloway, B. J. (2013) Heavy metals in soils: Trace metals and metalloids in soils and their bioavalibility. *3rd edition. Springer*.