



The Development of Blood Stain Removal Solution from Spinach in Longer Storage at Room Temperature for Sanitation Work in Hospitals

Nalin-on Nuiplot

Science Program of General Education, Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathum Thani, 13180, Thailand

Corresponding author. E-mail address: nalin-on@vru.ac.th

Received: 19 June 2020; Revised: 7 August 2020; Accepted: 17 August 2020

Abstract

The cleanliness in hospital is very essential for preventing of germ spreading especially from the secretion contaminated on clothes of patients such as saliva, lymph, and blood. Nowadays, two widely-used methods for the blood stain remover on patients' clothes are Hypochlorite using which contains the irritated chemical components, and Ozone disinfectant which is expensive and has a risk of ozone leaking causing accumulation and toxic to the body. However, a previous study had used the extract from spinach to test the blood stain remover. The spinach contains a large amount of oxalate which can be precipitated with metal ion (Ferrous ion in blood) and then be removed easily. Nevertheless, there are limitations in terms of time and place in storing the extract. This research therefore aims to develop blood stain removal solution from spinach by testing against detergent N70 to be conveniently stored and prolong the storage time in room temperature. The spinach samples were available from 3 areas in Thung Yai District, Nakhon Si Thammarat Province, Thailand. The solution from spinach was mixed with the solution of 500 g Chloride salt in 3 liters of water and 1 kg of the anionic surfactant substance; Sodium Lauryl ether sulfate (SLES) or Texapon N70 or N70. The mixed solution was tested with blood stained clothes which were donated from Ban Khlong Priang Subdistrict Health Promotion Hospital, Thung Yai District, Nakhon Si Thammarat Province, Thailand. The results showed that the developed blood stain remover from spinach has a storage period of up to 6 months; from normal: only 1 day at room temperature or 7 days at 4 degrees Celsius. The detergents also do not contain phosphate chemical resulting in being a non-toxic stain remover solution. Moreover, it is cost only 70 Bath per 1 liter then it can reduce the cost of buying stain remover solution about 3 times. As a result, it will help reduce the cost of purchasing blood stain remover which is a deeply stain and is often found in households and various medical institutions.

Keywords: Stain remover, Sanitary service, Blood stain, Oxalate, Spinach

Introduction

In daily life, there is many stains which are often found on clothes. Those common stains are either easy or difficult stains to eliminate, such as sweat stain, ink stain, oil stain, or even blood stain. The blood stain is commonly found in hospitals, especially from the patients, the elderly, naughty young children, or menstruation women. Blood stain on fabric are often taken a long time to remove and leads to health risks, namely the risk of infection from the blood and the condition of using unhygienic items. Although there are currently many products for the convenience of cleaning in many forms, but often has a high price and the main components are chemicals that both destroy fabric, destroy water resources, and often irritating to the skin. Common items that remove blood stains are vinegar, ammonia, hydrogen peroxide, cola, corn starch, talcum powder, and cold saltwater (Former University of Illinois Extension Educator, 2018; Mississippi State Department of Health, 2019; USDA Natural Resources Conservation Service, 2017; Jill, 2018; wikiHow staff, 2019). Moreover, some chemicals are harmful to the body, both in the form of acute allergies or accumulation of toxicity in the body (Thongmor, 2006).

There are very few published studies on the extracted from spinach and used to remove blood stains. Only the research of (Nalin-on, 2109), there was a study about the removal of blood stains by using extracts from the family of Amaranth (Amaranthaceae) (Stevens, 2017) which are spinach (*Spinacia oleracea*) and Amaranth (*Amaranthus viridis*). Both plants are easily found in the Thailand (Adul, 2019) and also in the study area. They contain the same chemical characteristics, especially oxalate substance (Somchai & Pennapa, 1996) which can be found up to 500 milligrams/100 grams of fresh weight (Narumon, 2014). The oxalate can bind to the metal in the body such as iron and calcium resulting in sedimentation until the formation of stones (Buonocore, 1955; Noonan & Savage, 1999; Yu & Ni, 2007). The properties of binding with the metal oxalate substances can be applied in removing blood stains from clothes because the blood contains hemoglobin (Hb) molecules. The main component of Hb is Iron (Ferrous) or Ferrous Ion (Fe^{2+}) which can bind with oxalate (C_2O_4) to be a water insoluble compound called Ferrous Oxalate or Iron Oxalate. (Iron (II) Oxalate), which has a molecular formula as FeC_2O_4 (s) and has a structural formula as shown in Figure 1.

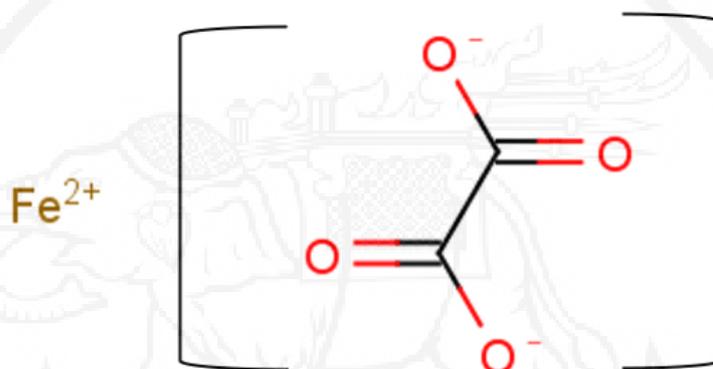


Figure 1 The structural formula of Iron oxalate (FeC_2O_4) (s) Reference: applied from VVchem, 2010

Within the area of Ban Khlong Priang, Thung Yai District, Nakhon Si Thammarat Province is a lowland area. In the rainy season, there will be floods that is suitable for rice, rubber and oil palm plantations. During the above-mentioned economic horticulture gardening, there was also planting of vegetables. One of them is spinach because it is easy to grow, easy to care for, can be cooked in a variety of food, and has a high nutritional value. In addition, the remaining spinach from cooking can be used as a laundry detergent that be focused on removing blood stains as well. Washing liquid from spinach extracts in the previous research (Nalin-on, 2019) there were still the restrictions on the storage time of the product as 1 day at room temperature and 7 days at a temperature of 4 degrees Celsius. Therefore, in this research, the researcher would try to find a helper or method to extend the storage time at room temperature of the blood stain removal solution from spinach. Using the least chemicals that do not cause irritation to the skin, and has the minimum effect on the environment, would be considered in this study. The expected from this research are 1) Blood Stain Remover from spinach that can remove blood stain, and contains low amount of chemicals and have a shelf life at room temperature for more than 1 day, and 2) To reduce the cost of buying blood stain remover which is often common deeply stain in hospitals.

The researcher therefore conducted a basic research on the preparation of various washing products. It was found that there are 2 substances that can be stored at room temperature for longer time than before and also



make a little skin irritation, and do not affect the environment. The first substance is the lye from ash (mostly from burning grains). By dissolving the ashes and leaving for 24 hours to precipitate, the clear water was used to soak the blood stain clothes and then be washed (Ernesto, 2019). The second substance is N70 inoculant that is inexpensive and can be easily purchased from general chemical stores. It is friendly to environment and can be used with every water condition (Rakbankerd, 2019). As a consequence, when considering the convenience both the time and the procurement of raw materials for production, the N70 was selected to extend the storage time at room temperature of the blood stain remover from spinach extract.

Research objective

1) To develop blood stain remover from spinach solution to be conveniently stored and prolong the storage time in room temperature by using against detergent N70

Methods and Materials

Research scope

1) In terms of content

This study is about making general stain remover and mixing the extract from spinach to help extend the life of the previous spinach extract product. Then, create a product to wash blood stains using spinach by using the least amount of chemicals

2) In terms of methods

Spinach extract in this study was obtained by applying the protocol of 2 studies: Nalin-on (2019) and Liu, Zhang, Li, Li, & Zhan (2015). In experiment of Liu, soluble oxalate was extracted from bamboo and Eucalyptus with water. As a result, in this study, 1 kg of spinach had been boiled (The temperature was around 70 °C.) in 600 ml of water for 180 min. This condition was indicated that it was an easy method and sufficient to completely extract the soluble oxalate as shown in Figure 2. After that, the boiled spinach solution was filtered with a thin white cloth. Before pouring the extracted solution in the prepared packaging, the solution can be used immediately to remove blood stains on fabric, or later mixed with the laundry detergent (N70) to extend the shelf life at room temperature.

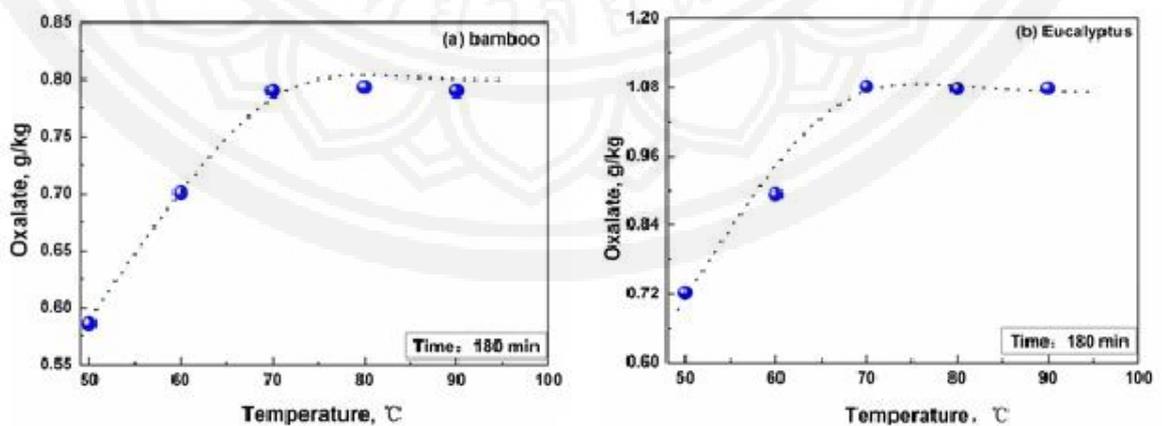


Figure 2 Effect of temperature on the extraction of soluble oxalate with water (a: bamboo; b: eucalyptus) Reference: Liu et al., 2015



N70 laundry detergent in this research was used only the solution of 500 grams of Chloride salt which diluted in 3 liters of water. Then the solution was poured into 1 kg of the anionic surfactant substance; Sodium Laurylether sulfate (SLES) or Texapon N70 or N70, (Koknis Thailand Company Limited). The substance was stirred before until it was turned a milky white color without adding color or smell. Then the detergent, which helps in removing blood stain and causes only a small amount of foam, was obtained.

Experiment methods

There are 1 sample group and 2 control group in this research as follows:

Group 1: Control group 1, 5 g of oxalate / 600 ml of water (+ve control)

Group 2: Control group 2, N70 detergent only

Group 3: Sample group, the extracted solution from boiled spinach and then be mixed with laundry detergent N70. The ratio of boiled spinach was 1 kg spinach: 600 ml of water. The plant samples in this study were derived from 3 areas; 1) "Tan Jai Bai Jai Saira" market, Ban Saira Subdistrict, 2) "Bang Roob" flea market, Bang Roob Subdistrict and 3) the planted spinach in farm land of Ban Khlong Priang Subdistrict. Those 3 areas were located in Thung Yai District, Nakhon Si Thammarat Province, Thailand. The spinach samples which were harvested 1-2 days before, were stored 1 day in the refrigerator and then extracted.

After spinning the boiled spinach, the spinach residues were filtered with a thin white cloth before filling the extracted spinach solution into the packaging. The extracted solution can be stored in the refrigerator before being mixed with laundry detergent N70. In each group of the experiments was repeated 3 times. For first step, 100 ml of pig blood was applied on a 30x30 cm thin white cloth. The second, the stain cloth were allowed to dry for 24 hours and then tested for blood stain removal. The next step, 100 ml of spinach extract was poured into water with the blood- stained cloth. Then the bloody clothes were soaked for 15 minutes before rubbing to get rid of the blood stain. After that, the results of all 3 experiments were repeated in each group to observe the efficacy of removing blood stains. The results were summarized as in Figure 3.

When the time limit was reached for soaking and washing, the clothes were unrolled to observe and compare the effectiveness of the blood stain removal of spinach extract in laundry detergent N70 with the control group. The remaining of the blood stain on cloth was observed as a following criteria of the color of the blood stain residual:

1) Remains up to the maximum amount more than 50% or the diameter of the blood stains is more than 5 cm, indicating that it is "less effective" in removing blood stains.

2) Remains up to the minimum amount less than 50% or the diameter of the blood is less than 2 cm, indicating that it is "effective" in removing blood stains.

The criteria of observing blood stains as shown in the Figure 4 were applied from the previous research (Nalin-on, 2019) and followed the effective indicator of Plaque index (Dental Health Promotion Group, 2012).

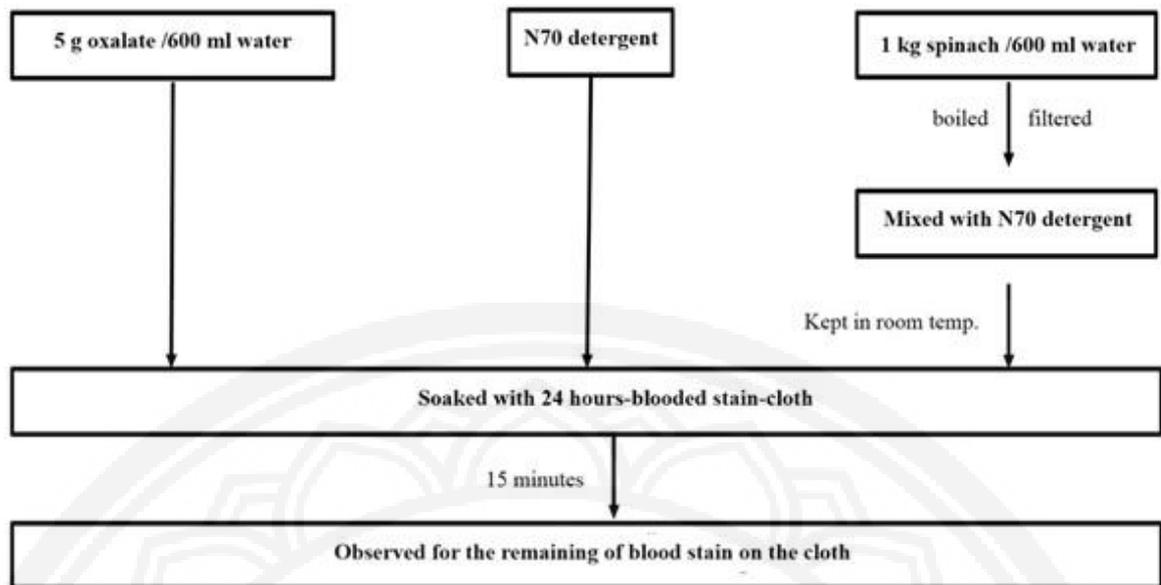


Figure 3 Diagram of experimental methods



Figure 4 The criteria of observing blood stain a) initial stains, b) less effective, and c) effective in removing Reference: Nalinon, 2019 and Ramita, Sirinapa, & Suthimapom, 2018

After the first blood test, the extracts of spinach mixed with laundry detergent N70 were kept at room temperature. In every month until the sixth month, they will be used for observing the test blood stain removal by using the experimental methods mentioned above.

Results

The result of blood stain observation was shown in Table 1.

Table 1 The average blood stain observation results

Times of experiment	Results of blood stain residual		
	Group 1	Group 2	Group 3
1	Less effective	Less effective	Effective
2	Less effective	Less effective	Effective
3	Less effective	Less effective	Effective

From table 1, the first to third time of blood stain removal experiments was showed that the blood stain in all 3 groups were able to remove although, in fact, the cleanliness or the size of the stain varied. In addition, even if keeping the bottle of spinach extract in N70 laundry detergent solution at room temperature for 6 months, it still has the ability to remove blood stains at a high level. The experimental results are shown in Figure 5-7.



Figure 5 Applied of 100 ml pig blood on each thin white cloth for 24 hours before testing



Figure 6 Results of groups 1, 2, and 3, respectively



Figure 7 The example of results by using this developed blood stain remover with hospital’s clothes after storing at room temperature in the 6th month (a: before testing, b: after testing)

The results in Figure 5– 6 could be analyzed to be the graph in Figure 8, when the X axis is the time of testing and the Y axis is the diameter of the remaining blood stains (centimeters) after washing in each group.

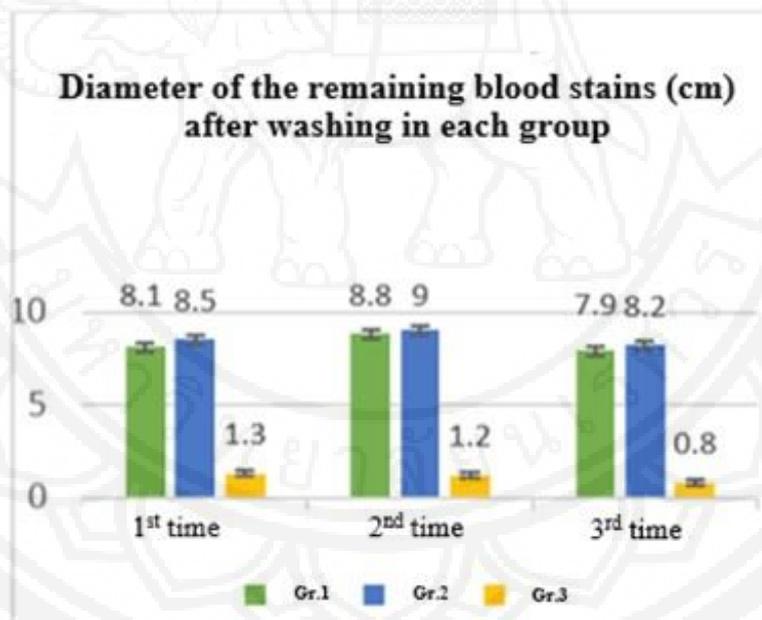


Figure 8 This bar chart shows the results of blood stain removal experiments. It was referred to the experimental data in Table 1 and Figure 5 (p-value <0.05)

Discussion

This research was aimed to develop the spinach stain remover from previous research (Nalin-on, 2019) to allow longer storage times at room temperature, and to reduce the cost of buying blood stain remover which is often common deeply stain in hospitals. From the above objectives, there will be led to reduce the risk of



infections in the hospital that may be caused by blood stains. This research was also tested with blood stained clothes from Ban Khlong Priang Subdistrict Health Promotion Hospital, Thung Yai District, Nakhon Si Thammarat Province, Thailand. There is a research report about the use of oxalate solution in the water of the clove leaf to remove blood stains on the fabric (Ramita et al., 2018). The research results were found that extracted clove leaf solution can remove blood stains on semi-synthetic and muslin fabrics. Because of oxalate binding to metal, the oxidation-reduction reaction occurs between the oxalate in the extracted clove leaf and iron or Ferrous (Fe^{2+}) in hemoglobin. They were then precipitated resulting in easily to eliminate the blood stains from cloth. However, in this research, spinach was used as a raw material for oxalate due to there is clear information about the amount of oxalate in spinach, and this plant can be widely found in the research area.

This research has developed the extracted spinach solution by mixing in laundry detergent N70 due to convenience both in terms of time and raw material sourcing. These reasons will lead to easily production for using in real conditions. The result of this research showed that the developed solution from spinach had more effective in removing blood stains than using only oxalate solutions or N70 detergent. The experimental results show that oxalate substances in Amaranth was able to bind to the metal (Ferrous iron in red blood cells for this research), and resulting in insoluble sediment which could help the blood stain to be removed from the fabric. In addition, the reinforcement with laundry detergent N70 made the blood stains be eliminated more easily. This experiment is considered the use of natural products, such as spinach, to be used to remove blood stain which is considered a kind of hard-to-remove stains. Although the extracted solution combined with the use of only a small amount of chemicals, namely salt and N70, it rarely affects the environment and does not cause skin irritation.

Conclusion and Recommendations

This research showed that there is the way to extend the life of the product or extracted solution from spinach comparing to the original product; only 1 day at room temperature or 7 days at a temperature of 4 degrees Celsius to be more than 6 months at room temperature. Because the substance N70 or the main component is SLES which is highly resistant to biodegradation, therefore the developed solution is not easily degraded. Moreover, it can be stored for 2 years or more under the conditions that is away from sunlight (Takayoshi et al., 2019). Therefore, although the color of the product will be slightly changed depending on the storage period but it is still effective in removing blood stains.

This developed formula is a new product of blood stain remover and more convenient to use than an extracted spinach solution without N70. Since once mixed, it will make the blood stain remover appear thicker, therefore there is not waste during using and do not need to apply directly to the fabric. It's also good value for money than buying blood stain removers in the market which are almost 3 times higher in price at the same volume. The initial cost of laundry detergent N70 leavening agent is 240 baht per 1 set. While other equipment can be used in household items, or if buying a new one, the total cost is not over 300 baht. Moreover, it can be produced as many as 3 liters of bottles from 1 set. In addition, the minimum volume required for each wash (10 pieces of clothing) is 100 ml of developed extracted spinach solution.



The extracted spinach solution that has been developed, can reduce the cost of purchasing blood stain remover and is suitable for using to eliminate the risks of infection from blood in hospitals.

1. The packaging should include a lid or spoon for measuring the removal solution for standardize of volume in each time of using.
2. It should have a food coloring or a natural color added to reduce the dullness of the blood stain remover solution when it had been storing for a long time.

Acknowledgments

Thank you to 10 students of Valaya Alongkorn Rajabhat University under the Patronage, Pathum Thani Province, which were this research assistants.

Thank you Ms. Kularb Moolasart, a professional nurse (Nursing), Professional level who donated the bloody clothes from Ban Khlomg Priang Subdistrict Health Promotion Hospital.

Thank you for General Education program, Valaya Alongkorn Rajabhat University under the patronage, Pathum Thani Province, which always supports and encourages students and teachers to constantly innovate.

Finally, thank you to the family for the encouragement and inner strength that has always been for me.

References

- Adul, C. (2019). *Spinach and Amaranth, the Local Vegetables with High Valuables*. Retrieved from https://www.technologychaoban.com/folkways/article_21454
- Buonocore, M. G. (1955). A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. *Journal of Dental Research*, *34(6)*, 849–853.
- Dental Health Promotion Group. (2012). *Identification of Knowledge*. Retrieved from http://km.hpc3.org/?wpfb_dl=65
- Ernesto, S. (2019). *Wood Ash as a Natural Cleaning Material*. Retrieved from <https://ecovillage.org/solution/wood-ash-as-a-natural-cleaning-material/>
- Former University of Illinois Extension Educator. (2018). *Stain Solutions*. Retrieved from <https://web.extension.illinois.edu/stain/staindetail.cfm?ID=5>
- Jill, N. (2018). *This is the Best Way to Remove Blood Stains from Fabric*. Retrieved from <https://www.onegoodthingbyjillee.com/how-to-remove-blood-stains/>
- Liu, Y., Zhang, C., Li, B., Li, H., & Zhan, H. (2015). Extraction and determination of total and soluble oxalate in pulping and papermaking raw materials. *BioRes*, *10(3)*, 4580–4587.
- Mississippi State Department of Health. (2019). *Green Cleaning Recipe*. Retrieved from <https://msdh.ms.gov/msdhsite/index.cfm/44,4900,360,pdf/GreenCleaning.pdf>
- Nalin-on, N. (2019). Study on blood stain removal using spinach. *UTK Research Journal*, *13(1)*, 109–115.
- Narumon, P. (2014). Crystal Calcium Oxalate and Oxalate Content in Some Vegetables in Nongkhai Province. *KKU Science Journal*, *42(4)*, 820–829.
- Noonan, S. C., & Savage, G. P. (1999). Oxalate content of foods and its effect on humans. *Asia Pacific Journal of Clinical Nutrition*, *8(1)*, 64–74.



- RakBanKerd. (2019). *Invest only 240 baht to clean the whole house (Easy-to-use multipurpose liquid)*. Retrieved from <http://rakbankerd.com/agriculture/highlight-view.php?id=16&s = tblheight>
- Ramita, M., Sirinapa, K., & Suthimaporn, N. (2018). *The effect of extract of Chaplo leaves on blood stain removal*. Phitsanulok: Science Project Upper Secondary Level, Biological Program, Chalermkwanstree School.
- Somchai, C., & Pennapa, A. (1996). "Spinach" (Amaranthu) (indigenous vegetables, precious nutrition). *Thammasat Journals*, 4(2), 45-57.
- Stevens, P. F. (2017). *Amaranthaceae family*. Retrieved from <http://www.mobot.org/MOBOT/research/APweb/>
- Takayoshi, K., Miki, T., Akihiro, M., Hayato, M., Tadayuki, T., Kohji, N., ... Sadanori, A. (2019). Fatty Acid Potassium Had Beneficial Bactericidal Effects and Removed *Staphylococcus aureus* Biofilms while Exhibiting Reduced Cytotoxicity towards Mouse Fibroblasts and Human Keratinocytes. *International Journal of Molecular Sciences*, 20(2), 312.
- Thongmor, J. (2006). *The production of bathroom cleaners from natural extracts*. Suphan Buri: Knowledge Pack, Development and Promotion of Organic Agriculture Learning Center, Regional Civil Society Development Institute.
- USDA Natural Resources Conservation Service. (2017). *Home and Garden Tips – Hazardous Products*. Retrieved from https://www.nrcs.usda.gov/wps/portal/nrcs/detail/national/home/?cid=nrcs143_023354
- VVchem. (2010). *Ferrous oxalate dihydrate*. Retrieved from [https:// http://www.vvchem.com/dictionary/en/6047-25-2.html](https://http://www.vvchem.com/dictionary/en/6047-25-2.html)
- wikiHow staff. (2019). *How to Remove Dried Blood Stains from Fabric*. Retrieved from <https://www.wikihow.com/Remove-Dried-Blood-Stains-from-Fabric>
- Yu, L., & Ni, Y. (2007). Partition of soluble and precipitated oxalate and its implication on decreasing oxalate-related scaling during peroxide bleaching of mechanical pulps. *Pulp and Paper Canada*, 108(1), 39-43.