Effect of Particle Size, Solvent and Extraction Time on Tannin Extract from *Spondias purpurea* Bark Through Soxhlet Extraction

Hilary Y. Baldosano, Ma. Beatriz Micaela G. Castillo,
Chantal Danica H. Elloran, Florinda T. Bacani*
Chemical Engineering Department, De La Salle University, 2401 Taft Ave., 1004 Manila
*Corresponding author: florinda.bacani@dlsu.edu.ph

Abstract: In this study, the tannin content of *Spondias purpurea* or siniguelas bark was explored due to the lack of information and studies on the species. This study aimed to investigate the effects of particle size, solvent type and extraction time on the extraction of tannin from its bark through Soxhlet extraction. The ratios of solvent used were ethanol-water solutions at 0:1, 1:1, and 0.5:9.5. The extraction time was also varied at 4, 6, and 8 hours. The extracts were then analyzed using a Perkin Elmer High-Performance Liquid Chromatography (HPLC) with UV/VIS detector. The HPLC was performed using a reversed phase C-18 column as the stationary phase, and methanol (Solvent A) and 1:25 aqueous acetic acid solution (Solvent B) as the mobile phase. It was confirmed that extraction parameters varied in this study affected the yield of tannin in the extract. Finer particle sizes can produce a higher yield of tannin as long as agglomeration in the extractor does not occur. Moreover, a longer extraction time produces a higher yield. Solvents with higher amount of ethanol gave better yields of tannin. Pure water, as a solvent, gave the lowest amounts of tannin due to the solubility of proteins; the 95% ethanol was the most effective. It was observed that 95% ethanol with the longest extraction time of 8 hours gave the highest percentage yield of 19.19% and 17.13% from 10 grams of bark. The model generated that gave an insignificant lack of fit was quadratic. Using ANOVA, the P-value obtained from the model was less than 0.0001, which implies that it is significant.

Key Words: tannin; solvent extraction; soxhlet extraction; *Spondias purpurea*; High-Performance Liquid Chromatography (HPLC);

1. INTRODUCTION

Tannins are phytochemical polyphenols found in most plants and trees, particularly in their bark and wood (Steiner, 1989). They possess several beneficial characteristics such as being astringent, an antioxidant, and anti-bacterial (Sher, 2004). They are have a wide range of uses in industries such as the leather tanning, textile dyeing, ink, wood adhesive, and wine industries. Their ability to interact with and precipitate proteins, such as proteins found in animal hides, are what make them important raw materials in industries.

Since ancient times, tannins have been used in the traditional craft of textile dyeing as one of these mordants. Other examples of natural mordants include tannic acid, tartaric acid, guava and banana leaves and bark (Mathur & Gupta, 2003). However, as the industry expanded and more advanced technologies were developed, the use of natural materials in the dyeing industry decreased. The advancement of technologies led to the development of synthetic mordants such as alum, copper sulfate, and ferrous sulfate, which are relatively cheaper and more reproducible (Bechtold et al., 2003). However, many sources and experts believe that the use of such synthetic materials cause several health risks and environmental pollution (Forgacs et al., 2004). In modern times, the apparent need for safe and environment-friendly materials have re-established the significance of natural mordants in the textile dyeing industry (Mathur & Gupta, 2003).

In the Philippines, dyes, whether synthetic or natural, have usually been imported into the country rather than manufactured (PTRI, 2005). The Philippine Statistics Authority (PSA) has published in its August 2014 Preliminary External Trade Performance Report that the country's imports of dyeing, tanning and coloring materials is a US $29.01 million commodity. Also, the report showed that the annual growth rate of the commodity from 2013 to 2014 was 11.5%. To reduce the country's heavy reliance on synthetic dye imports and to establish local natural dyeing technologies, the Department of Science and Technology's Philippine Textile Research Industry (PTRI) conducted a study in 2005 that identified 26 indigenous plant species as promising sources of natural dyes and one of them was *Spondias purpurea*, known as sineguelas (PTRI, 2005).

Sineguelas or *Spondias purpurea*, from the *Anacardiaceae* family, is a fruit-bearing tree mostly found in the tropics such as South and Central America, Africa, and Asia (Bicas et al. 2011). It is also commonly known as the spanish plum, jocote, ovo, ciruela, or siriguela in other countries. The family where *Spondias purpurea* belongs to, *Anacardiacea*, is considered to be one of the tannin-rich Dicotyledons (Mahlo & Chauke, 2012). The recent discovery of the plant's natural dyeing abilities could be due to its tannin content. According to a study on natural dyeing, the ability of plant to act as a natural dye or biomordant could be attributed to its high tannin content (Prabhu et al., 2011). Furthermore, a study on the tannin content of the leaves of the plant affirms that both plant parts contain tannins (Asaad et al., 2006). Several sources have also indicated the presence of tannin in its bark, which is known to be a primary source of tannin (Steiner, 1989). Thus, this study focused on the extraction of tannin from the plant's bark through Soxhlet extraction, which is an effective method in extracting phenolic compounds (Roberts et al., 2013). In addition, the effect of the extraction parameters particle size, solvent type, and extraction time on the yield of tannin was investigated.

2. METHODOLOGY

2.1 Collection and sample preparation

The plant used for this experiment was obtained from Barangay Ulingan, Bagac, Bataan. A branch of the tree was brought to the National Museum of the Philippines for taxonomic identification. Phytochemical test was done by Standards and Testing Division of the Department of Science and Technology to determine the present constituents in the bark sample, as well as to confirm the presence of tannin. The bark was freeze dried to determine its moisture content. The freeze-dried bark was then ground into the desired particle sizes using the laboratory mill. The weight of the bark sample used per run was 10 grams.

After determining the moisture content, Soxhlet extraction of the bark samples were performed. The factors observed in the extraction were the type of solvent and the extraction time. The crude extracts obtained were then analyzed in the HPLC. After acquiring the results of the HPLC, the percentage yields of each were calculated and the data were analyzed using the Design Expert Program.

2.2 Soxhlet extraction

Each run used 10 grams of bark with a particle size of -20+48 mesh, which was determined in the preliminary experiment. The solvents used, were distilled water, 50% ethanol (v/v) or 95% ethanol (v/v) with a constant volume of 300 ml. The solvent were heated up until it reached its boiling point. The extraction process was conducted for 4 hours, 6 hours and 8.

The solvent used and the extraction time were varied. The responses obtained were the concentration of the sample and the percentage yield. The number of runs was 9 with 2 trials, adding up to a total of 18 runs. The interpretation of the General Factorial design is that for example for the first run the values that will be used for the solvent and extraction time are 50% ethanol and 6 hours. Also, the response of the design was the percentage yield.

3. RESULTS AND DISCUSSION

3.1 Percentage Yield of Tannin

The results of the first trial, as shown on Figure 1, determined 95% ethanol as the solvent ratio that gave the highest percentage yields for the extraction times of 4, 6, and 8 hours with 3.60%, 8.26%, and 19.19%, respectively. On the other hand, extraction with water resulted to the lowest percentage yields of 2.23%, 2.45%, and 3.96% for 4, 6, and 8 hours. Using 50% ethanol gave percentage yields that were in between those of water and 95% ethanol.

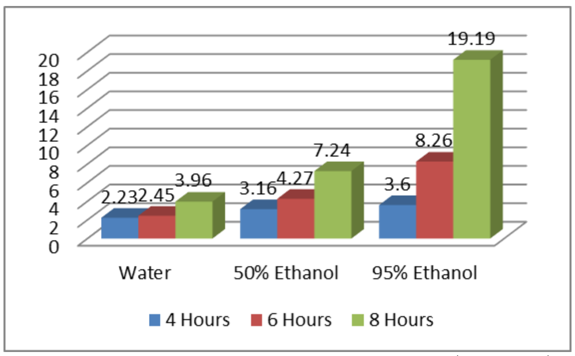

Fig. 1 Percentage Yields of Samples (1st Trial)

The results of the second trial, shown on Figure 2, also determined that a solvent ratio of 95% ethanol with an extraction time of 8 hours gave the highest percentage yield of 17.13%. However, for the extraction times of 4 and 6 hours, the use of 95% ethanol only gave percentage yields of 2.76% and 6.49%. Rather, it was 50% ethanol that gave the highest percentage yields of 4.14% and 6.73% for extraction times of 4 and 6 hours. Water gave the lowest percentage yields of 2.16%, 2.51%, and 3.31% for extraction times of 4, 6, and 8 hours.

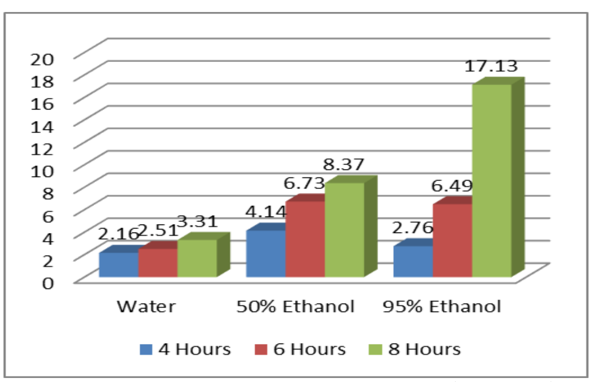

Fig. 2 Percentage Yields of Samples (2nd Trial)

The results of the percentage yields are summarized in Table 2; while, Figures 1 and 2 shows the graph of the percentage yield as a function of the type of solvent and extraction time.

Table 2 Percentage Yield of the Samples

Solvent	Time (hour)	Percentage Yield (%)
50% ethanol	6	4.27
95% ethanol	8	19.19
Water	6	2.45
Water	4	2.23
95% ethanol	4	3.60
95% ethanol	6	8.26
Water	8	3.96
Water	6	2.51
50% ethanol	8	7.24
50% ethanol	4	3.16
Water	8	3.31
95% ethanol	4	2.76
50% ethanol	6	6.73
95% ethanol	6	6.49
50% ethanol	8	8.37
50% ethanol	4	4.14
95% ethanol	8	17.13
Water	4	2.16

As expected, a longer extraction time generally led to a higher percentage yield of tannin. This could have been due to the longer amount of time the solute and solvent were in contact with each other. Longer contact time favored the system to have more mass transfer. However, excessive extraction time would be unnecessary as the solvent and sample would be in final equilibrium after certain duration. This is based on Fick's second law of diffusion. By then, the rate of extraction of compounds would decelerate (Tan et al. 2013).

Moreover, a higher percentage yield was also observed for solvents that had higher percentages of ethanol. Although tannins are highly soluble in water, water proved to be an ineffective extraction solvent for tannins. This could be due to the formation of tannin-protein complexes. The effectiveness of ethanol as an extraction solvent for tannin could be due to it being an organic polar solvent. The polarity of ethanol makes it able to have strong interactions with polar substances such as tannin. Also, the results actually affirms the theory that extracting at high temperatures could degrade and lose some phenolic compounds ("Quantification of Tannins in Tree Foliage," 2000). Since of all the solvents used, 95% ethanol had the lowest boiling point, it was able to extract the most tannin. Moreover, the result was in accordance with other previous studies stating that mixture of alcohols and water as the solvent could extract phenolic compounds better compared to mono-solvents like pure water (Dent, 2012; Spigno et al. 2007; Tomsone et al. 2012).

Also, based on the results of the preliminary experiment, it was found that extracting with smaller particle sizes could produce a higher yield. Smaller particle sizes offer greater surface area for mass transfer. Finer particles, however, are more prone to agglomeration. Even if 0.297mm (-48 mesh) was the smallest particle size used in the study, it did not give the highest yield of tannin. The solvent might have had a hard time passing through the sample due to its compactness which resulted to low extraction efficiency.

3.2 Analysis using Response Surface Methodology

The Design Expert Program was used in order to analyze the data acquired. A probability of less than 0.05 shows that the lack of fit is significant. An insignificant lack of fit means that there is no need to look for a higher order type of model. In this case, the only model with an insignificant lack of fit is quadratic with a probability value of 0.0515.

Analysis of Variance (ANOVA) was done to test the validity of the model as well as whether the factors, solvent and time of leaching, observed have a significant effect on the percentage yield of tannin. The F-value of the model is 44.10 which implies that it is significant. Also, the probability of it occurring due to noise is only 0.01%. The values of the "Prob > F" should be less than 0.05 for the model terms to be considered as significant. Since the values are less than 0.0001, the model terms (A, B, AB) are significant.

From the ANOVA results, the final equations of the model were generated: Equation (1) is for the water as the solvent, Equation (2) is for 50% ethanol, and Equation (3) is for 95% ethanol.

$$\%Yield=11.60306-(3.5975*Time)+(0.32979*Time^2) \quad (1)$$
$$\%Yield=10.41222-(2.9187*Time)+(0.32979*Time^2) \quad (2)$$
$$\%Yield=-1.90528-(0.2125*Time)+(0.32979*Time^2) \quad (3)$$

The normal plot of residuals can be seen in Figure 3. The points on the graph represent the normal percentage probability of the percentage yields with respect to the residuals. It can be seen that the points are approximately linear. Since the plot of residuals fit the expected pattern, it shows that the residuals are distributed normally. On the other hand, Figure 4 shows the plot of externally studentized residuals versus predicted values. Since the points are in random and show no pattern, the model is suitable to the data. It also satisfies the independent normally distributed residuals that are usually assumed (Hoerl, 2008).

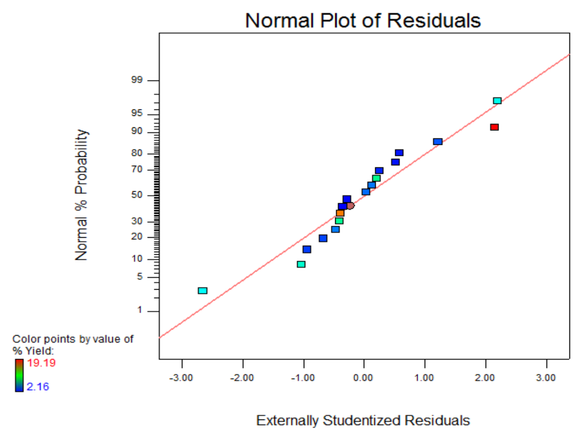

Fig. 3 Normal Plots of Residuals

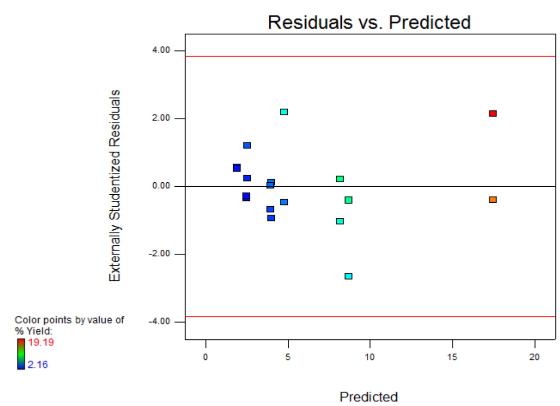

Fig. 4 Residuals versus Predicted Graph

Figure 5 shows the graph of the interactions of the factors, type of solvent and time of leaching, observed in the study. It can be seen that at 4 hours, the percentage yield points are overlapping. There is no type of solvent that actually stands out for that specific time. However, for the times 6 and 8 hours, the 95% ethanol solvent performed well compared to the other solvents.

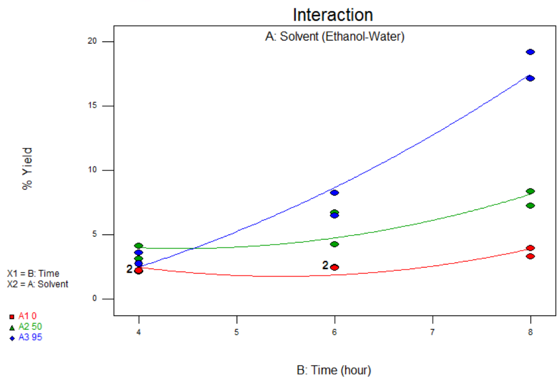

Fig. 5 Interactions of the Factors

4. CONCLUSIONS

Even though finer particle sizes offer greater surface area for mass transfer, it does not guarantee a higher yield of tannin. Finer particle sizes are more prone to agglomeration which could hinder the extraction process, but larger particle sizes are also ineffective. Thus, the most effective particle size for extraction should be able to maximize the surface area for mass transfer, yet prevent agglomeration.

It was confirmed that the factors, extraction time and type of solvent, investigated in this study do affect the yield of tannin in the extract. Statistical analysis showed that there are significant interactions between the factors and the response. The result in this study is in agreement with the previous studies stating that as the extraction time increases, the extraction yield also increases in various solvents. A longer extraction time would give the bark and solvent better equilibrium and mass transfer.

Comparing the three solvents used, water was the least effective in extracting the tannin due to the solubility of proteins in water. The solvent that gave the highest percentage yield of tannin was 95% ethanol.

The optimum conditions that afforded the highest percentage yield of 19.19% (Tannic Acid Equivalent) were 95% ethanol solvent and 8 hours of extraction time. It was known that the bark of *Spondias purpurea* has a higher tannin content compared to its other parts.

5. REFERENCES

Asaad, C. O., De Vera, A. C., Ignacio, L. M., & Tigno, P. B. (2006). Evaluation of lesser-known and lesser-utilized feed resources in the Philippines. Improving Animal Productivity by Supplementary Feeding of Multinutrient Blocks, Controlling Internal Parasites and Enhancing Utilization of Alternate Feed Resources, 239

Bechtold, T., Turcanu, A., Ganglberger, E., & Geissler, S. (2003). Natural dyes in modern textile dyehouses—how to combine experiences of two centuries to meet the demands of the future. Journal of Cleaner Production, 11(5), 499-509.

Bicas, J., Molina, G., Dionísio, A., Barros, F. C., Wagner, R., Maróstica, M., et al. (2011).Volatile constituents of exotic fruits from Brazil. Food Research International, 44(7), 1843–1855. Retrieved January 10, 2013, from http://www.sciencedirect.com/science/article/pii/S096399691100024X

Dent, M., Uzelac, V., Penić, M., Brnčić, M., Bosiljkov, T., & Levaj, B. (2012). The Effect of Extraction Solvents, Temperature and Time on the Composition and Mass Fraction of Polyphenols in Dalmatian Wild Sage (Salvia officinalis L.) Extracts. Food Technology and Biotechnology. Retrieved March 21, 2013, from http://www.ftb.com.hr/index.php/component/content/article/133-volume-51-issue-no-1/1076-the-effect-of-extraction-solvents-temperature-and-time-on-the-composition-and-mass-fraction-of-polyphenols-in-dalmatian-wild-sage-salvia-officinalis-l-extracts

Forgacs, E., Cserhati, T., & Oros, G. (2004). Removal of synthetic dyes from wastewaters: a review. Environment international, 30(7), 953-971

Hoerl, R. (2008). Statistics Roundtable: The Reality of Residual Analysis. Retrieved November 7, 2014, from http://asq.org/quality-progress/2008/06/statistics-roundtable/the-reality-of-residual-analysis.html

Mahlo, S., & Chauke, H. (2012). The effectiveness of polyethylene glycol (PEG) and polyvinyl polypyrrolidone (PVPP) on removal of tannins from leaf extracts of selected medicinal plants in Limpopo Province. African Journal of Biotechnology, 11(43), 10141-10145. Retrieved December 2, 2012, from http://www.academicjournals.org/ajb/P

DF/pdf2012/29May/Mahlo%20and%20Chauke.pdf

Mathur, J. P., & Gupta, N. P. (2003). Use of natural mordant in dyeing of wool. Indian Journal of Fibre and Textile Research, 28, 90-93.

Philippine Statistics Authority (PSA). (2014). External Trade Performace: August 2014. Retrieved Novemer 13, 2014 from http://web0.psa.gov.ph/content/external-trade-performance-august-2014

Philippine Textile Research Institute (PTRI). (2005). The Philippine Scene: 26 Dye-Yielding Trees Identified. Textile Developments, 20(2), 0118-34X. Retrieved from http://faculty.mu.edu.sa/public/uploads/1345909499.9763TD_Ap-Je05.pdf

Prabhu, K., & Bhute, A. (2012). Plant based natural dyes and mordants: A Review.Journal of Natural Product and Plant Resources, 2(6), 649-664

Prabhu, K., & Teli, M. (2011). Eco-dyeing using Tamarindus indica L. seed coat tannin as a natural mordant for textiles with antibacterial activity. Journal of Saudi Chemical Society. Retrieved April 5, 2014, from http://0-www.sciencedirect.com.lib1000.dlsu.edu.ph/science/article/pii/S131961031100202X

Prabhu, K., Teli, M., & Waghmare, N. (2011). Eco-friendly dyeing using natural mordant extracted from Emblica officinalis G. Fruit on cotton and silk fabrics with antibacterial activity. Fibers and Polymers, 12(6), 753-759

Quantification of Tannins in Tree Foliage. (2000). Joint Fao/Iaea Division Of Nuclear Techniques In Food And Agriculture. Retrieved November 9, 2014, from http://www-naweb.iaea.org/nafa/aph/public/pubd31022manual-tannin.pdf

Roberts, T., Wilkes, W., & Khoddami, A. (2013). Techniques for Analysis of Plant Phenolic Compounds. Molecules, 18, 2328-2375

Sher, A. (2004).Antimicrobial activity of natural products from medicinal plants. Gomal Journal of Medical Sciences , 7(1). Retrieved March 2, 2013, from http://gjms.com.pk/ojs786/index.php/gjms/article/download/165/164

Spigno, G., & De Faveri, D. (2007). Antioxidants from grape stalks and marc: Influence of extraction procedure on yield, purity and antioxidant power of the extracts.Journal of Food Engineering, 78(3), 793–801-793–801. Retrieved November 3, 2014, from http://www.sciencedirect.com/science/article/pii/S026087740500779X

Spigno, G., Tramelli, L., & De Faveri, D. M. (2007). Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. Journal of Food Engineering, 81(1), 200-208. Retrieved March 21, 201, from http://www.sciencedirect.com/science/article/pii/S0260877406006649

Steiner, P. R. (1989). Tannins as specialty chemicals: An overview. In Chemistry and Significance of Condensed Tannins (pp. 517-523). Springer US.

Tan, M., Tan, C., & Ho, C. (2013). Effects of extraction solvent system, time and temperature on total phenolic content of henna (Lawsonia inermis) stems.International Food Research Journa, 20(6), 3117-3123. Retrieved November 12, 2014, from http://www.ifrj.upm.edu.my/20%20(06)%202013/21%20IFRJ%2020%20(06)%202013%20Ho%20446.pdf

Tomsone, L., Kruma, Z., & Galoburda, R. (2012). Comparison of Different Solvents and Extraction Methods for Isolation of Phenolic Compounds from Horseradish Roots (Armoracia rusticana). World Academy of Science, Engineering and Technology, 6, 1155-1160. Retrieved November 11, 2014, from http://waset.org/publications/8584/comparison-of-different-solvents-and-extraction-methods-for-isolation-of-phenolic-compounds-from-horseradish-roots-armoracia-rusticana-