Philippine Journal of Science
151 (5): 1579-1588, October 2022
ISSN 0031 - 7683
Date Received: 13 Jan 2022

Phytochemical Analysis and Antibacterial Potential of Tabon-tabon (*Atuna racemosa* Raf) Fruit Extract against *E. Coli* BIOTECH 1634

Christine Ann S. Tila[1*], **Lotis E. Mopera**[2], **Aimee Sheree A. Barrion**[3], and **Katherine Ann C. Israel**[2]

[1]National Fisheries Research and Development Institute, Corporate 101, Mo. Ignacia Avenue, South Triangle, Quezon City 1105 Philippines
[2]Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna 4031 Philippines
[3]Institute of Human Nutrition and Food, College of Human Ecology, University of the Philippines Los Baños, College, Laguna 4031 Philippines

This study aimed to examine the phytochemical levels of crude "tabon-tabon" (*Atuna racemosa* Raf.) fruit extracts subjected to air-drying, oven-drying, and freeze-drying. It also sought to validate the claims of previous literature on the fruit's antimicrobial properties by using its aqueous extracts as an antibacterial wash for green ice leaf lettuce. Phytochemical analysis revealed significantly higher ($p < 0.05$) levels of alkaloids and tannins compared to coumarins, saponins, and terpenoids. The highest alkaloid content (16.25 ± 1%) was obtained using oven-drying, whereas the highest tannin content was obtained using freeze-drying (14.14 ± 1.13%). *In vivo* antibacterial assay against *Escherichia coli* was performed to test the aqueous extracts alongside 200 ppm chlorine solution and water for comparison. Artificially-inoculated lettuce samples were individually soaked in 15-mL solution at 2- and 5-min contact times. Bacterial load was determined at a 2-d interval for a period of 8 d. Results of the microbiological analysis showed no significant interaction in the soaking time used. However, the treatments were independently found to have significant effects on Days 0, 4, and 6. The highest antibacterial effect in green ice lettuce was exhibited up to Day 6.

Keywords: alkaloids, antibacterial, *Atuna racemosa*, phytochemicals, "tabon-tabon"

INTRODUCTION

Atuna racemosa Raf., known as "tabon-tabon" in the Philippines, is an indigenous canopy tree alleged with promising antimicrobial properties (Buenz *et al.* 2007; Feitosa *et al.* 2012). It is native to selected regions in Mindanao and can grow up to 45 m tall. The fruit is commonly used as an ingredient in a traditional dish called *kinilaw*. In other countries, the tree is commonly harvested from the wild for local use as a medicine and as a source of materials, especially as a caulk for boats (Fern 2014). The extract from the grated flesh of the fruit is typically used to neutralize the fishy taste and the acidity of the raw seafood dish (Davidson 2014). This prehistoric fruit has not been considered edible upon ripening due to its hard flesh, which is also found with a strong astringent flavor. It belongs to the Crysobalanaceae

*Corresponding author: cstila@up.edu.ph

family, where several plants have also been reported to have promising active components (Feitosa 2012). In 2019, a study was conducted by Nadayag *et al.* on the antibacterial properties of the inner bark of *Atuna racemosa* samples. The conducted antibacterial assay showed positive antibacterial effects against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa*. In addition, aqueous, ethyl acetate, methanol and decocted extracts of the shell and kernel of *A. racemosa* showed positive activity against *E. coli* and *S. aureus*. Despite having limited use in the Philippines (Fang-asan *et al.* 2009), proven antibacterial properties of these plant species suggest their potential use as antimicrobial agents.

Natural antimicrobials have found their use in fresh-cut vegetables as washing solutions. This was found to be a viable means of inhibiting spoilage or avoiding oxidative processes. Its effects are recognized with safer effects compared to traditional food preservatives such as chlorine, organic acids, and calcium-based solutions, which cause allergic reactions in sensitive individuals, as well as the formation of potentially carcinogenic by-products (Rico *et al.* 2007).

Drying methods, as mentioned in several research works, produce significant results on the preservation of natural phytochemicals. In a study conducted by Barimah *et al.* (2017), freeze-drying and solar drying were found to have lower adverse effects on the antioxidant capacity of dandelion (*Taraxacum officinale* L.) leaves. Natural products offer great opportunities for finding novel bioactive compounds with potential bioactivities. Thus, plant extracts offer a significant role in the discovery of bioactive compounds. However, it is important to establish the scientific rationale to defend their use in many applications.

In this regard, the study seeks to provide baseline information on the quantitative levels of phytochemicals found in the crude extracts of *Atuna racemosa* fruit as affected by various drying methods. It also aims to validate claims on the antibacterial properties of the plant by testing its aqueous fruit extracts (FEs) against *E. coli* artificially inoculated on green ice leaf lettuce.

MATERIALS AND METHODS

Collection of Plant Samples

Hand-picked samples of ripe *A. racemosa* fruits were collected from Maramag, Bukidnon in the Philippines. The fruit was identified by the locals in the area and was verified in terms of physical characteristics according to the work of Pacaña and Galarpe (2017). The samples were placed in polyethylene bags and were stored at room temperature until further use.

Preparation of Crude Plant Samples Using Different Drying Techniques

The acquired *A. racemosa* fruits were thoroughly cleaned by washing with running tap water and subsequently with a 200-ppm chlorinated solution to remove adhering dirt and contaminants. Then, the samples were left to dry at ambient temperature for 1 h. Whole fruits were cut into half and the flesh was scraped off. The fruit flesh was subjected to air-drying at 25 ± 3 °C, oven-drying (using the DCM Stationary Gas Oven 3DG2) at 45 ± 3 °C, and freeze-drying at –30 °C (using the DOST-developed Bench-top Freeze Dryer) until a constant weight was attained. The dried samples were then pulverized using a bullet blender and were sieved (40 mesh) to ensure a uniform particle size. The percent recovery was recorded, and the crude plant powders were stored in airtight amber bottles and were kept in a desiccator until further use.

Quantitative Determination of Phytochemicals in Crude Plant Extracts

Quantitative phytochemical estimates were conducted using the methods detailed below.

Alkaloids. The method presented by Ezeonu and Ejikeme (2016) was used to quantify the alkaloid content of the crude plant powders. One (1) g of crude *A. racemosa* powder was placed in a beaker and was added with 40 mL of 10% acetic acid in ethanol. The glassware was covered and left to stand at room temperature for 4 h. Then, it was filtered, and the filtrate was concentrated using a water bath (Yamato Scientific Co., Ltd. 168026) until a quarter of its original volume was obtained. Subsequently, concentrated ammonium hydroxide was added dropwise to the extract until complete precipitation was achieved. The solution was left at room temperature overnight to allow the complete settling of the precipitate. This was washed with dilute ammonium chloride (1%) and was filtered. The residue was dried to obtain a constant weight, and the total alkaloid content was expressed mathematically as:

$$\%\ alkaloids = \frac{Weight\ of\ residue}{Weight\ of\ crude\ powder} * 100 \qquad (1)$$

Tannins. Total tannins were determined using the Folin-Ciocalteu method as described by Makkar (2003), wherein 1 g of crude *A. racemosa* powder was boiled in 400 mL of distilled water for 30 min. The solution was transferred to a 500-mL volumetric flask and was diluted to the mark. The resulting solution was mixed well and filtered. Then, an

aliquot (0.02 ml) of the same solution was placed in screw-capped test tubes. Distilled water was added to make up the volume to 0.5 mL. Then, 0.25 mL of the Folin-Ciocalteu reagent and 1.25 mL of 20% sodium carbonate solution were added. The resulting solution was vortexed and was allowed to settle at room temperature for 40 min. The absorbance of the standards and samples were read at 725 nm using a UV-Vis spectrophotometer (UV-Mini-1240). The total amount of tannic acid was computed using the calibration curve generated from the absorbance of the standard solutions. Total tannins (%) was expressed as tannic acid using the equation:

$$\%\ tannins\ as\ tannic\ acid = (C)\left(\frac{Volume\ of\ solution}{Weight\ of\ crude\ powder}\right)\left(\frac{1\ g}{1\ x\ 10^6\ \mu g}\right)(DF) * 100 \quad (2)$$

C = concentration of tannic acid computed using linear regression (µg/mL)

Saponins. The quantitative determination of saponins was performed according to the work of Ejikeme *et al.* (2014) with modifications. Twenty (20) g of crude *A. racemosa* powder was placed in a beaker and was added with 100 mL of 20% aqueous ethanol. The samples were heated over a hot water bath (Yamato Scientific Co., Ltd. 168026) for 4 h with continuous stirring at 55 °C. The mixture was filtered, and the residue was re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL over a water bath at 90 °C. Then, the concentrate was transferred into a 250-mL separatory funnel, added with 20 mL of diethyl ether and was vigorously mixed. The aqueous layer was recovered, whereas the ether layer was discarded. This purification process was repeated twice. Then, 60 mL of n-butanol was added to the recovered solution. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. Then, the remaining solution was heated in a water bath. After evaporation, the samples were dried in an oven (Memmert UM300) to constant weight; the saponin content was calculated as a percentage using the equation:

$$\%\ saponin = \frac{Weight\ of\ saponin\ residue}{Weight\ of\ crude\ powder} * 100 \quad (3)$$

Terpenoids. Total terpenoids of plant samples were determined according to the work of Indumathi *et al.* (2014). One hundred (100) mg of crude *A. racemosa* powder was measured and soaked in 9 mL of ethanol for 24 h. Then, the ethanolic extracts were filtered and extracted with 10 mL of petroleum ether using a separatory funnel. The ether extract was separated in pre-weighed glass vials and was allowed to dry completely. Ether was evaporated and the yield (%) of total terpenoids was computed using the equation:

$$\%\ terpenoids = \frac{Weight\ of\ residue}{Weight\ of\ crude\ powder} * 100 \quad (4)$$

Coumarins. The total coumarin content of the crude *A. racemosa* powder was determined by measuring the absorbance of the yellow solution as described by Pacaña and Galarpe (2017). The concentration of coumarin was computed using its molar absorptivity in ethanol at 311 nm, 5700 L mol^{-1} cm^{-1} (Taniguchi and Lindsey 2018). Ethanol extracts were prepared by soaking 0.5 g of powdered samples in 50 mL of ethanol for 24 h. An aliquot (1 mL) of this solution was re-dissolved in 50 mL of the solvent. Then, 10% NaOH was added to 1 mL of the diluted solution. The formation of yellow color indicates the presence of coumarin in the solution. The absorbance of the samples was measured using a UV-VIS spectrophotometer (UV-Mini-1240) at 311 nm. The final concentration of coumarin in the solution was computed using Beer's Law, and the total percentage in the crude sample was determined using the equations below:

$$C = \frac{A}{\varepsilon\ b} \quad (5)$$

where:
A = absorbance at 311 nm
ε = molar absorptivity
b = path length
C = concentration of coumarin

$$\%\ coumarins = \frac{(C)(Volume\ of\ solution)(MW\ of\ coumarin)(DF)}{Weight\ of\ crude\ powder} * 100 \quad (6)$$

where:
C = concentration of coumarin
Volume of solution = 0.5 L
MW coumarin = 146.1427 g/mol
DF = dilution factor
Weight of crude powder = 0.5

Preparation of Aqueous Plant Extracts

One hundred (100) g of crude *A. racemosa* powder was dissolved in 500 mL of sterile distilled water and was soaked for 24 h with constant agitation for the first 6 h. Then, the extract was filtered through a Buchner funnel using a vacuum pump. The collected filtrate was freeze-dried (DOST-developed Bench-top Freeze Dryer) at –30 °C for 72 h to obtain a fine powder. The freeze-dried powder was weighed, stored in airtight amber bottles, and placed in a desiccator until further use. The total extraction yield was computed using the equation:

$$\%\ extraction\ yield = \frac{Weight\ of\ lyophilized\ powder}{Weight\ of\ crude\ powder} * 100 \quad (7)$$

***In Vivo* Antibacterial Testing of Aqueous FE**

Collection of lettuce samples. Green Ice leaf lettuce samples were purchased from South Supermarket, Los Baños, Laguna, Philippines. These were placed in a styrofoam box maintained at 5–7 °C and were transferred in a chiller not more than 12 h prior to analysis.

Preparation of test microorganism. Pure cultures of *Escherichia coli* (BIOTECH 1634) were provided by the Institute of Food Science and Technology, University of the Philippines Los Baños (IFST, UPLB). Single colonies from stock cultures were obtained by streak plating. These were transferred in plate count agar (PCA) slants and were incubated at 35 ± 2 °C for 24 ± 4h. Then, the acquired pure cultures were stored at 5 ± 3 °C until further use.

Preparation of E. coli inoculum. Prior to use, *E. coli* stock cultures were sub-cultured twice in tryptic soy broth (TSB) contained in centrifuge tubes at 35 ± 2 °C for 24 ± 4h. The latter tubes were centrifuged at 4 °C (4,500 rpm) for 15 min and were subsequently washed and re-suspended in 0.1% peptone water. The suspensions were pooled to obtain a 500-mL bacterial cocktail.

Preparation of washing treatments. Three washing solutions (sterile distilled water, 200 ppm chlorine solution, and aqueous *A. racemosa* FE) were used as treatments in the study. Sterile distilled water was prepared by sterilizing distilled water at 121 °C for 15 min. Chlorine solution, on the other hand, was prepared using commercially available bleach. For the plant extract, 100 g of oven-dried aqueous extract powder was dissolved in 500-mL sterile distilled water. The solution was left to soak for 24 h and was filtered using a sterile cheesecloth. The filtrate was stored in a sterile media bottle prior to use.

Preparation of lettuce samples. Lettuce samples were inspected to ensure no wilt, rotten, or damaged part. Healthy lettuce leaves were individually cut to 6 cm^2 using a surface-sterilized knife and chopping board. Then, the tissue cuts were surface sterilized by soaking in 10% NaOCl solution for 2 min, followed by two sets of sterile distilled water, each for 1 min. Lettuce samples were blot dried in a sterile improvised tray lined with sterile tissue paper and were left to dry under the hood for 30 min.

Soaking of lettuce samples in different sanitizing treatments. Surface sterilized lettuce samples were dipped in a 500-mL bacterial cocktail for 1 min and were placed in sterile wire baskets in a laminar flow hood for 2 h to allow proper attachments. Using sterile distilled water and 200 ppm chlorinated solution as control treatments, the samples were dipped in each sanitizing treatment for 2 and 5 min, each in three replications. These were carried out by soaking leaf tissues in 15 mL of each sanitizing solution for the allotted time. After soaking, these were placed in sterile Petri plates and incubated at 5–7 °C.

Enumeration of E. coli in lettuce samples. Lettuce samples were collected for microbial analysis at different storage intervals (Days 0, 2, 4, 6, and 8). These were individually placed in sterile plastic bags with 100 mL of 0.1% peptone water and were homogenized in a 400-circulator stomacher at 230 rpm for 2 min. Several dilutions were prepared, and the appropriate dilutions for each sample were plated on violet red bile agar (VRBA) in triplicates and were incubated for 24 ± 4h at 35 ± 2 °C. Colonies were counted from the three trials conducted, and the values were reported as CFU/cm^2 using the equation:

$$\frac{CFU}{cm^2} = \frac{(\sum c)(DF)}{[(1\ x\ N_1) + (0.1\ x\ N_2)](d)} \quad (8)$$

where:
N = number of colonies per mL or g of product
$\sum C$ = sum of all colonies on all plates counted
n_1 = number of plates in first dilution counted
n_2 = number of plates in second dilution counted
d = dilution from which the first counts were obtained
DF = dilution factor of homogenate

Statistical Analysis

The experiments in this study were conducted using a factorial completely randomized design. All analyses were done in triplicates and were subjected to analysis of variance using the SPSS 16.0 software and SAS Software Version 12. The difference between treatment means was determined using the least significant difference test, declared at a 95% confidence interval (Weaver *et al.* 2018).

RESULTS AND DISCUSSION

Phytochemical Composition of Tabon-tabon

Effect of different drying methods on the phytochemical composition of tabon-tabon. Constant weight obtained from the samples marked the end of the drying period. Time lengths required for the complete drying of plant materials vary depending on the drying technique used. Air-drying contributed to a longer drying time (120 h) compared to oven-drying (72 h) and freeze-drying (8 h). Figures 2 and 3 show that drying methods influence drying and aqueous extraction yield. Oven-drying preserved the highest water-soluble phytochemicals, as expressed by

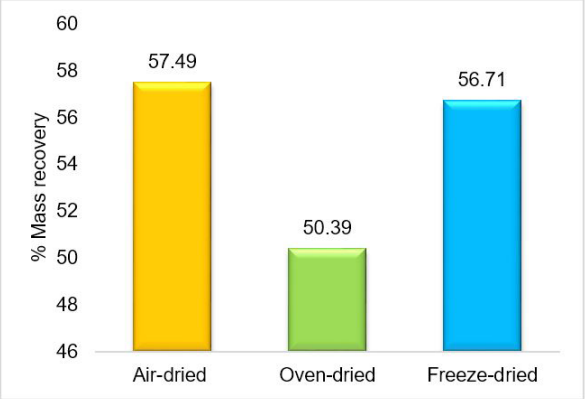

Figure 2. Mass recovery (%) of crude plant material after air-drying (37± 3 °C), oven-drying (45 ± 3 °C), and freeze-drying (–30 °C).

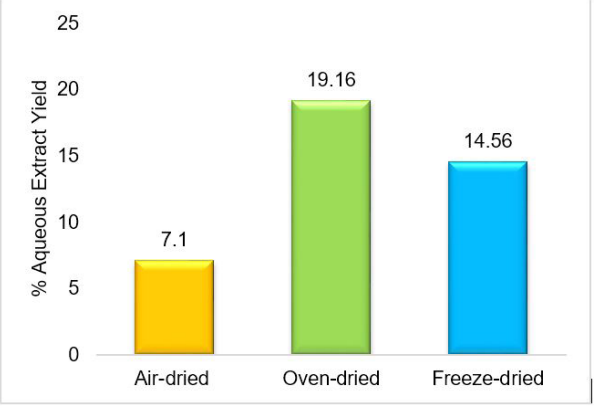

Figure 3. Aqueous fruit extract yields after lyophilization at –30 °C.

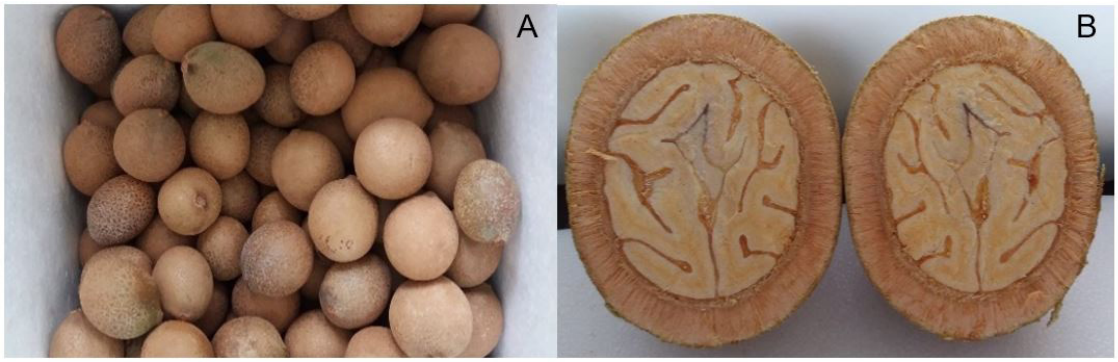

Figure 1. Whole (A) and cross-section (B) of tabon-tabon (*Atuna racemosa*) fruit.

the extraction yield. Crude plant powder, however, was lower compared to air-dried and freeze-dried samples. It could be due to higher water evaporation, which reduces the bulk of the plant material (Pham *et al.* 2015).

In a study conducted by Pacaña and Galarpe (2017), phytochemicals present in aqueous *A. racemosa* extracts were qualitatively examined. The said study confirms the presence of alkaloids, saponins, tannins, terpenoids, and coumarins. Thus, it was used as baseline information on the quantified phytochemicals in this study. Results on the total yield of phytochemicals, as summarized in Table 1. Alkaloids were significantly higher when oven-drying was used compared to the other drying methods. On the other hand, significantly higher amounts of tannins were retained with the use of freeze-drying. Other phytochemicals examined were found in significantly lower amounts. These results show that crude *A. racemosa* powder contains significant amounts of alkaloids and tannins. However, the selection of the drying method is essential depending on the compound of interest.

According to Azwanida (2015), air-drying usually takes from 3–7 d to months depending on the type of samples dried. This drying method does not force dried plant materials using high temperatures; hence, heat-labile compounds are preserved. However, the length of time devoted to drying exposes the plant materials to contamination and unstable temperature conditions. Aside from this, oxygen exposure upon drying results in increased redox activity and degradation of phenolic compounds (Pham *et al.* 2015). Higher levels of alkaloids and tannins obtained using oven-drying and freeze-drying, respectively, could mean that they are more efficient drying processes for these phytochemicals. Freeze-drying operates through the process of sublimation, wherein a solid is changed into the gas phase without entering the liquid phase, thus preserving high-value compounds. It has also been proven to be an efficient method in retaining plant bioactive compounds because dehydration occurs at lower temperatures. However, it may not be cost-effective since it is a complex and expensive method of drying compared to regular drying methods (Mediani *et al.* 2015). Contrary to the claim of various researchers, higher alkaloid levels in this study were obtained using oven-drying. According to Orphanides *et al.* (2013), thermal processing – especially oven drying – promotes the breakdown of bioactive compounds, as attributed to the activity of degradative enzymes such as polyphenol oxidases (PPOs), which can degrade phenolic compounds but, oven drying at lower temperatures proved to be efficient in preserving high phytochemical levels in this study. This is also in agreement with the work of Oduje and John (2015), wherein phytochemical constituents of plant samples were best preserved at 40 °C compared to 80 °C.

Effect of aqueous FEs on the bacterial load of lettuce. Having the highest extraction yield, the oven-dried aqueous extract was selected for *in vivo* antibacterial analysis. Lettuce, one of the most commonly consumed fresh vegetables lasts at least a week under refrigerated storage conditions (FDA 2010). Hence, it was chosen as the commodity for testing the effect of FE.

FE was compared to water and 200 ppm chlorine by determining the bacterial reductions on artificially inoculated lettuce samples with varying soaking times for 8 d. Samples were drawn on Days 0, 2, 4, 6, and 8 for microbial analysis and checking of appearance. No interaction between the sanitizing solution and soaking time was observed. Thus, a soaking time of either 2 or 5 min has the same level of effect on the log population of coliforms per day. The treatments were found to have significant effects on the bacterial population on Days 0, 4, and 6.

Figure 4 illustrates the antibacterial effects of *A. racemosa* FE on green ice leaf lettuce upon storage. On Day 0, It had significantly lower effects when compared to chlorine. Although it was significantly more effective than water, it did not show similar antibacterial properties to chlorine. However, on Day 4, an increase in the antibacterial effects of FE can be seen, as evidenced by the reduced bacterial population. It was able to produce the same

Table 1. Phytochemical content of crude plant powder using different drying methods.

[1]Treatment	Phytochemical content (%)				
	Alkaloids	Saponins	Tannins	Terpenoids	Coumarins
AD	4.57 ± 2.20^{bZ}	1.23 ± 0.88^{cX}	6.53 ± 0.49^{aZ}	1.43 ± 0.15^{cY}	1.41 ± 0.05^{cX}
OD	16.25 ± 1.79^{aX}	1.14 ± 0.10^{cX}	9.31 ± 0.43^{bY}	3.54 ± 0.91^{cX}	2.59 ± 0.19^{cX}
FD	12.00 ± 0.67^{bY}	0.93 ± 0.41^{cX}	14.14 ± 1.13^{aX}	2.47 ± 0.34^{cXY}	1.94 ± 0.19^{cX}

[1]AD = air-dried; OD = oven-dried; FD = freeze-dried
Values are recorded as means of three determinations, n = 3
[a–c]Means with different superscripts in the same row differ significantly ($p < 0.05$)
[X–Z]Means with different superscripts in the same column differ significantly ($p < 0.05$)

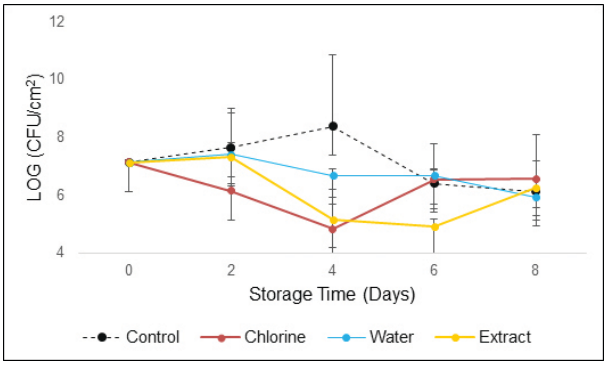

Figure 4. Effect of different sanitizing treatments on the total coliforms on lettuce stored at 5–7 °C.

level of protection as the treatments using chlorine wash. Improvements in the effects of FE can be seen on Day 6, as opposed to chlorine, since it significantly lowered the bacterial population among the three washes. Thus, it was able to prevent bacterial growth.

Effect of sanitizing solutions on population growth of E. coli. Among the sanitizing treatments employed, chlorine was found to have a rapid effect in reducing the bacterial population of lettuce samples contrary to water and plant extract during the early storage period but its effects diminished over time. As explained by Fouad (2017), the antibacterial effect of chlorine results from the hydrolysis product, hypochlorous acid (HOCl) which is formed from the reaction between the chlorine molecule and water:

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^-$$

Hypochlorous acid, a weak acid, can ionize as follows:

$$HOCl \rightleftharpoons H^+ + OCl^-$$

The degree of ionization depends primarily on the pH and temperature of the water. The effect of sodium hypochlorite on bacteria and tissue remnants is explained by several chemical reactions. HOCl and OCl- formed in the chlorine solution react with proteins, resulting in their degradation and hydrolysis. Upon reaction of HOCl with organic tissue, chlorine ions are released, which combine with amino groups of the protein molecules to chloramines, and interfere with bacterial metabolism. In addition, sodium hypochlorite alters bacterial membrane permeability by degrading the fatty acids in the bacterial cell membranes. Hypochlorous acid and hypochlorite ion are effective in killing bacteria. However, they will also oxidize reduced organic ions such as iron (II), manganese (II), and nitrite ion – as well as organic impurities. These compounds are collectively known as the chlorine demand. Thus, sufficient chlorine must be added to kill bacteria to exceed the chlorine demand (Kotz *et al.* 2012). By law, the maximum concentration allowed for sanitizing fresh produce is 200 ppm (USFDA 2014). The decreasing effects of chlorine against bacteria have also been observed in several related studies. Rodgers *et al.* (2004) explained that this could be due to the reaction with plant tissues and extracellular biochemical components, which renders chlorine ineffective to inactivate bacterial cells attached to or embedded in plant tissue. In addition, the hydrophobic structure of the waxy cuticle of fruit and vegetable skins provides a natural barrier to bacterial attachment and penetration in flesh tissue. Moreover, higher efficacy of chlorine was observed in vegetables with less structural complexity. The plant extract, on the other hand, showed greater effects in reducing the bacterial population at prolonged storage time. This could be attributed to the bioactive components identified earlier in this study. According to Su *et al.* (2015), active substances of plant extracts act on the bacterial cell membrane or outer membrane proteins, leading to the destruction of bacterial cell growth. Cowan (1999) also explains that the polyphenol antimicrobial mechanism may be related to the inhibition of hydrolytic enzymes (proteases) or other interactions that inactivate microbial adhesions, cell envelope transport proteins, and non-specific interactions with carbohydrates. In another study, chemical compounds examined from crude ethanolic extracts of spice were found to affect multiple target sites against the bacterial cells (Burt 2004; Oonmetta-aree *et al.* 2006). Among the extracts used, water was found to be the least effective, although it was able to decrease bacterial population at certain periods, washing with water alone was not enough to reduce bacterial growth in Green Ice lettuce upon storage.

Effect of sanitizing solutions on the physical appearance of lettuce. The physical appearance of food is one of the many factors contributing to consumer acceptability and food quality (Barrett *et al.* 2010). Water created minimal changes in the appearance of lettuce as opposed to chlorine and plant extract, which caused gradual changes in leaf tissues over time (Figure 5). Adverse effects on lettuce tissue treated with chlorine were observed on Day 6. Compared to the early days of storage, tissue became soft and darker in color. This damaging chemical effect suggests application at lower concentrations or shorter storage time.

The plant extract, on the other hand, was observed to cause darkening on lettuce tissue on Day 6, which further developed into dark spots on Day 8. This could be caused by a high concentration of plant extract used. In a study conducted by Joshi *et al.* (2016), the darkening of vegetables was also noted upon the application of plant-based antimicrobial wash. Among the leafy vegetables evaluated, the darkest color reaction was noted on lettuce. This could also be due to prolonged contact with plant

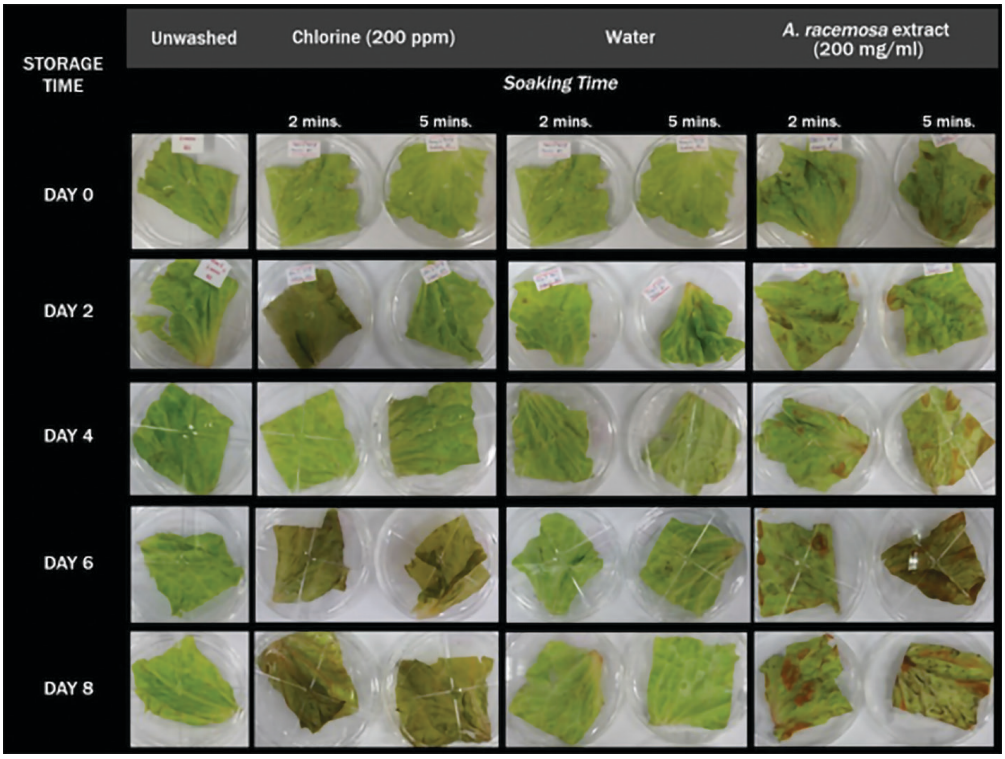

Figure 5. Appearance of representative lettuce samples after incubation at refrigerated temperature.

extracts, which were previously examined with significant amounts of phenolic compounds. Since lettuce treated with water alone did not cause any darkening reactions to the lettuce tissue, the effects of browning could be due to substances present in the extracts.

According to Holderbaum *et al.* (2010), the darkening of lettuce tissue could be a result of enzymatic reactions. Enzymatic browning is one of the reactions that lead to negative changes in the color of fruits and vegetables. This reaction is a consequence of the phenolic compound's oxidation by PPO, which triggers the generation of dark pigments. Polyphenols, which were earlier found to be present in the plant extracts, are reactive compounds that could influence PPO activity. In the presence of oxygen, PPO oxidizes mono- and di-phenols to o-quinones. Once the products are formed, they polymerize to form high molecular weight compounds or brown pigments, as they are highly reactive. The brownish color could be enhanced when they react with amino acids and proteins. Aside from this, discoloration could also be attributed to the high concentration of antimicrobial substances.

Asare *et al.* (2018) observed concentration-dependent changes in minimally processed fresh lettuce upon application of the antimicrobial wash, acrolein. It was observed that higher concentrations of the antimicrobial wash effectively reduced the plate count of microorganisms until Day 7. However, it induced pronounced discoloration of lettuce. Hence, it is important to determine the optimum time and plant extract concentration that results in positive effects on both microbiological and physical characteristics of leafy vegetables. Unwashed lettuce maintained a good physical appearance during extended storage time, despite having a high bacterial load. Thus, a good physical appearance does not necessarily mean that the produce is free of bacteria. In this regard, washing and sanitizing before storage are vital for food safety. Based on the results obtained, FE proved to be effective in reducing the log population of bacteria in lettuce tissue. However, high concentrations might not apply to leafy vegetables at longer storage time since they are more sensitive and are prone to suffer from rapid enzymatic browning.

CONCLUSION

This study proved that different drying techniques significantly affect the yield of crude powder and the amount of phytochemicals present in the fruit flesh of *A. racemosa.* Among the three, a higher drying yield was obtained from air-dried and freeze-dried samples. However, for aqueous extraction, more water-soluble

extracts were recovered from oven-dried samples with a 19.16% yield. Predominant compounds present in dried samples were alkaloids and tannins. However, appropriate drying techniques for individual phytochemicals differed. Oven-drying and freeze-drying methods were most effective for alkaloids (16.25%) and tannins (11.32%), respectively. Saponins (0.93–1.23%), terpenoids (1.43–3.54%), and coumarins (1.41–2.59%), on the other hand, were found in relatively lower amounts.

Having the highest yield, aqueous oven-dried plant extracts were used for the *in vivo* antibacterial assay. A contact time of 2 and 5 min did not show any difference in the treatment effects. FE was found to effectively reduce the log population of *E. coli* in lettuce at longer storage time. However, the concentration used did not favor good physical appearance on lettuce tissue over time, as evidenced by the appearance of dark spots. FE resulted in more effective antibacterial performance during longer storage as opposed to chlorine, which declined in effects over time. With the results obtained, it can be deduced that FE contains significant amounts of phytochemicals responsible for its antibacterial properties. Due to the adverse effects of aqueous *A. racemosa* extract on lettuce tissue at prolonged storage, it is recommended to determine the appropriate concentration that could reduce bacterial action and preserve the overall quality of vegetables at the same time.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Department of Science and Technology–Science Education Institute the UPLB-IFST for the financial assistance and the facilities provided throughout the course of this study.

REFERENCES

ASARE P, GREPPI A, STETTLER M, SCHWAB C, STEVENS M, LACROIX C. 2018. Decontamination of minimally-processed fresh lettuce using reuterin produced by *Lactobacillus reuteri*. Front Microbiology 9: 1421.

AZWANIDA N. 2015. A review on the extraction methods use in medicinal plants, principle, strength, and limitation. Medicinal and Aromatic Plants 4(3): 3–8.

BARRETT DM, BEAULIEU JC, SHEWFELT R. 2010. Color, flavor, texture, and nutritional quality of fresh-cut fruits and vegetables: desirable levels, instrumental and sensory measurement, and the effects of processing. Critical Reviews in Food Science and Nutrition 50(5): 369–389.

BARIMAH J, YANNEY P, LARYEA D, QUARCOO C. 2017. Effect of drying methods on phytochemicals, antioxidant activity, and total phenolic content of dandelion leaves. American Journal of Food and Nutrition 5(4): 136–141.

BUENZ EJ, BAUER BA, SCHNEPPLE DJ, WAHNER-ROEDLER DL, VANDELL AG, HOWE CL. 2007. A randomized Phase I study of *Atuna racemosa*: a potential new anti-MRSA natural product extract. Journal of Ethnopharmacology 114(3): 371–376.

BURT S. 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology 94(3): 223–253.

COWAN MM. 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews 12(4): 564–582.

DAVIDSON A. 2014. The Oxford Companion to Food. New York, USA: Oxford University Press.

EJIKEME CM, EZEONU CS, EBOATU AN. 2014. Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta area of Nigeria. European Scientific Journal 10(18): 247–270.

EZEONU CS, EJIKEME CM. 2016. Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwoods. New Journal of Science.

FANG-ASAN M, DAGUPEN M, DIGAL L. 2009. Lettuce Supply Chains and Marketing Margins in Benguet, Philippines. BANWA Archives (2004–2013) 6(2): 32–42.

[FDA] Food and Drug Administration. 2010. Program Information Manual Retail Food Protection: Recommendations for the Temperature Control of Cut Leafy Greens during Storage and Display in Retail Food Establishments. Retrieved on 14 Nov 2018 from https://www.fda.gov/downloads/Food/GuidanceRegulation/RetailFoodProtection/UCM218902.pdf

FEITOSA EA, XAVIER HS, RANDAU KP. 2012. Chrysobalanaceae: traditional uses, phytochemistry, and pharmacology. Revista Brasileira de Farmacognosia 22(5): 1181–1186.

FERN K. 2014. *Atuna racemosa* raf. Chrysobalanaceae. Useful Tropical Plants Database 2014. Retrieved on 10 Jul 2017 from http://tropical.theferns.info/viewtropical.php?id=Atuna+racemosa

FOUAD A. 2017. Endodontic Microbiology, 2nd ed. John Wiley and Sons.

HOLDERBAUM DF, KON T, KUDO T, GUERRA MP. 2010. Enzymatic browning, polyphenol oxidase

activity, and polyphenols in four apple cultivars: dynamics during fruit development. Hort Science 45(8): 1150–1154.

INDUMATHI C, DURGADEVI G, NITHYAVANI S, GAYATHRI PK. 2014. Estimation of terpenoid content and its antimicrobial property in *Enicostemma litorrale*. International Journal of ChemTech Research 6(9): 4264–4267.

JOSHI K, SPARKS P, FRIEDMAN M, RAVISHANKAR S. 2016. Impact of Plant-Based Antimicrobial Washes on Sensory Properties of Organic Leafy Greens. Food and Nutrition Sciences 7(10): 906.

KOTZ JC, TREICHEL PM, TOWNSEND J. 2012. Chemistry and chemical reactivity. California, USA: Cengage Learning. 956p.

MAKKAR H. 2003. Measurement of Total Phenolics and Tannins Using Folin-Ciocalteu Method. In: Quantification of Tannins in Tree and Shrub Foliage. Vienna, Austria: Springer, Dordrecht. p. 49–50.

MEDIANI A, ABAS F, KHATIB A, TAN CP, ISMAIL IS, SHAARI K, ISMAIL A, LAJIS NH. 2015. Relationship Between Metabolites Composition and Biological Activities of *Phyllanthus niruri* Extracts Prepared by Different Drying Methods and Solvents Extraction. Plant Foods for Human Nutrition 70(2): 184–192.

NADAYAG J, DAPAR MLG, ARANAS AT, MINDO RAR, CABRIDO CK, MANTING MME, DEMAYO CG. 2019. Qualitative assessment of the antimicrobial, antioxidant, and phytochemical properties of the ethanolic extracts of the inner bark of *Atuna racemosa*. Pharmacophore 10(1): 52–59.

ODUJE A, JOHN A. 2015. The effect of different drying temperatures on the nutritional composition and phytochemical constituents of *Jatropha multifida* leaves. Der Pharmacia Sinica 6(2): 80–84.

OONMETTA-AREE J, SUZUKI T, GASALUCK P, EUMKEB G. 2006. Antimicrobial properties and action of galangal (*Alpinia galanga* Linn.) on *Staphylococcus aureus*. LWT-Food Science and Technology 39(10): 1214–1220.

ORPHANIDES A, GOULAS V, GEKAS V. 2013. Effect of drying method on the phenolic content and antioxidant capacity of spearmint. Czech Journal of Food Sciences 31(5): 509–513.

PACAÑA J, GALARPE V. 2017. Antibacterial property of *Atuna racemosa* Rafin. *Chrysobalanaceae* shell and kernel extracts. International Journal of Biosciences. 11(1): 443–448.

PHAM H, NGUYEN V, VUONG Q, BOWYER M, SCARLETT C. 2015. Effect of extraction solvents and drying methods on the physicochemical and antioxidant properties of *Helicteres hirsuta* Lour. Leaves. Technologies 3(4): 285–301.

RICO D, MARTIN-DIANA AB, BARAT JM, BARRY-RYAN C. 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: a review. Trends in Food Science & Technology 18(7): 373–386.

RODGERS SL, CASH JN, SIDDIQ M, RYSER ET. 2004. A comparison of different chemical sanitizers for inactivating *Escherichia coli* O157: H7 and *Listeria monocytogenes* in solution and on apples, lettuce, strawberries, and cantaloupe. Journal of Food Protection 67(4): 721–731.

SU PW, YANG CH, YANG JF, SU PY, CHUANG LY. 2015. Antibacterial activities and antibacterial mechanism of *Polygonum cuspidatum* extracts against nosocomial drug-resistant pathogens. Molecules 20(6): 11119–11130.

TANIGUCHI M, LINDSEY JS. 2018. Database of Absorption and Fluorescence Spectra of > 300 Common Compounds for Use in PhotochemCAD. Photochemistry and Photobiology 94(2): 290–327.

[USFDA] United States Food and Drug Administration. 2014. Lettuce: FDA Investigation Summary – Multistate Outbreak of *E. coli* O157:H7 Illnesses Linked to Ready-to-eat Salads. Retrieved on 12 Aug 2017 from https://www.fda.gov/food/recallsoutbreaksemergencies/outbreaks/ucm374327.htm

WEAVER K, MORALES V, DUNN GODDE K, WEAVER P. 2018. An Introduction to Statistical Analysis in Research: with Applications in the Biological and Life Sciences. John Wiley and Sons Inc.