International Food Research Journal 22(1): 88-95 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Functional and antimicrobial properties of bignay [*Antidesma bunius* (L.) Spreng.] extract and its potential as natural preservative in a baked product

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Article history

Received: 15 March 2014
Received in revised form: 16 June 2014
Accepted: 8 July 2014

Keywords

Antimicrobial
Antioxidant
Bignay
Functional
Natural Preservative
Total phenolic

Abstract

The potential of bignay [*Antidesma bunius* (L.) Spreng.] fruit extract as a source of natural preservative was investigated. Bignay fruits, at red and fully mature stage, were analyzed for total phenolic content and free radical scavenging antioxidant activity. Crude and ethanol extracts from bignay fruits at concentrations 25%, 50%, 75% and 100% were screened for antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fluorescens, Bacillus subtilis*, *Candida albicans*, and *Aspergillus ochraceus* using paper disc diffusion assay. Efficacy of the extract in controlling microbial growth in muffin was examined from 0 to 6 d of storage. Sensory evaluation of the baked product was also performed. Fully mature bignay fruit extract has higher total phenolic content (141.80 g CE 100 g-1) and antioxidant activity (87.10%) than red bignay, 84.43 g CE 100 g-1 and 75.87%, respectively. Largest zones of inhibition were produced by ethanol (16.50-25.00 mm and 15.83-25.17 mm) and crude (13.67-21.67 mm and 15.50-23.83 mm) extracts from red bignay against *P. fluorescens* and *B. subtilis,* respectively. Red bignay extracts with pH adjusted to 6.5 also exhibited antibacterial effects against *P. fluorescens* and *B. subtilis*. Addition of 5% bignay extract can significantly inhibit the growth of microorganisms in a baked product and result in softer and more acceptable baked product. Bignay can be used as a natural ingredient with significant biological function due to its antioxidant and antimicrobial properties.

Introduction

Chemical preservative, as defined by the US Food and Drug Administration, "is any chemical that, when added to food, tends to prevent or retard deterioration thereof, but does not include common salt, sugars, vinegars, spices, or oils extracted from spices, substances added to food by direct exposure thereof to wood smoke, or chemicals applied for their insecticidal or herbicidal properties." These substances have played significant role in prolonging the shelf-life of foods and food supply considering the marketing system where foods are processed in one area and are often transported to several other areas for distribution. Consequently, there have been significant changes in the food market trends for the past years. There is increasing demand for foods that contain less synthetic preservatives. In order to respond to this emerging consumer demand, the food industry has extended efforts in eliminating or reducing synthetic additives and utilizing natural or nature-identical food preservatives (Fellows, 2000).

The growing market for more natural foods promoted the investigation of alternative food preservatives from natural sources. Among these sources are plants that contain high amounts of bioactive compounds such as essential oil, phenolics and organic acids. These substances have been linked to antioxidant property and antimicrobial activities. For instance, Wu *et al*. (2008) determined the antibacterial effects of American cranberry *(Vaccinium macrocarpon)* concentrate on foodborne pathogens, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus* in vitro and found out that the bioactive compounds of the cranberry concentrate influenced the functionality and antibacterial effects. Meanwhile, Katalinic *et al*. (2010) investigated the polyphenolic composition, related antioxidative and antimicrobial properties of grape skin extracts against Gram-positive and Gram-negative bacteria. Their results proved high antioxidant activity of extracts in terms of radical-scavenging and metal-chelating ability and effective antimicrobial action with minimum inhibitory concentrations of 0.014–0.59 mg of gallic acid equivalents (GAE)/ml.

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In the Philippines, several tropical fruits have been explored for their content of bioactive components, significant properties and constituents that could impart nutritional and health benefits to humans. Bignay [*Antidesma bunius* (L.) Spreng.], is one of the fruits that have been investigated for its biological activity, flavonoids and phenolic contents. Methanolic extracts from bignay berries were examined for anthocyanin, flavonoids, and phenolic acids constituents and antioxidant capacity to determine their potential as a source of natural antioxidants for foods and beverages (Butkhup and Samappito, 2008). Nevertheless, there is a lack of literatures describing the potential antimicrobial properties in relation to the bioactive components and functional properties of bignay. This commodity contains a large quantity of biologically active substances that could inhibit various metabolic activities of bacteria, yeast, and molds, yet, many of them are not fully exploited.

This study intended to (1) determine and compare the phenolic content and free-radical scavenging antioxidant activity of bignay fruit extract at red and fully mature stage, (2) evaluate the antimicrobial activity of bignay fruit extract against selected pathogenic and spoilage microorganisms using paper disc diffusion assay and (3) determine the efficacy of bignay fruit extract as a natural preservative in muffin.

Materials and Methods

Sample preparation

Bignay [*Antidesma bunius* (L.) Spreng.] berries, at the red mature and fully matured stage, were harvested in mid-September and mid-October, respectively, at the Institute of Food Science and Technology. The berries were washed, sorted and frozen at -4 °C until used and analyzed.

Extraction of polyphenols

The polyphenolic constituents of freeze dried homogenized berry was extracted using conventional solvent extraction procedure. For 10 g of sample, 100 ml of alcoholic solvent (ethanol/water 80/20, v/v) was added and extraction was done at 60°C for 60 minutes. After the extraction, samples were filtered with Whatman No. 1 filter paper and the residual matter was washed twice with 25 ml of solvent. The filtrates were combined in a total extract and concentrated by vacuum rotary evaporator at 50°C (Katalinic *et al*., 2009). The concentrated extract was weighed and kept at 4°C until used in the analysis.

Determination of total phenolic content

Homogenized sample extracts weighing 50 mg each were dissolved in 5.0 mL absolute methanol. The mixtures were shaken for 30 minutes in an evapomixer. The resulting mixtures were centrifuged at 3000 rpm for 5 minutes. In a test tube, 0.2 ml supernatant was transferred and was added with 2.8 mL distilled water followed by thorough shaking. Sodium carbonate with a concentration of 0.2 M and with a volume of 1.0 mL was then added. The resulting mixture was added with 0.2 mL Folin-Ciocalteu Phenol reagent and was mixed thoroughly. The test tubes containing the mixture were placed in a boiling water bath for 15 minutes and were cooled to room temperature. Absorbance was measured using UV spectrophotometer at 710 nm.

Standard curve was constructed using geometric concentrations of catechin stock solution. The slope from the regression line of the standard curve was obtained and the total phenol content of the samples was calculated using the formula:

where:
weight of sample = (50 mg/5mL) x (2.00 mL) = 20 mg

Analysis of antioxidant property

The antioxidant activity in terms of free radical-scavenging ability of bignay fruit extract was determined by the use of a stable 2,2-diphenyl-2-picrylhydrazyl radical (DPPH*). Approximately 2 g of each sample was used for the analysis. Each sample was placed in a test tube and was added with 20 mL of 50% methanol solution. Filtration was done using Whatman No. 1 filter paper. The extract was collected and placed in a clean vial with cover. The process was repeated for at least five times until the solvent became colorless. The vials were kept in at 4°C until use.

DPPH radical scavenging activity of bignay extract was measured according to the method of Shimada *et al*. (1992). An aliquot of each sample amounting to 1 mL was acquired and placed in a test tube. Each sample was added with 4.0 mL distilled water and mixed well. One mL of freshly prepared 1mM DPPH methanolic solution was added in the mixture. DPPH methanolic solution was prepared by dissolving 4 mg of DPPH in 100 mL absolute methanol. After the addition of DPPH solution, the resulting solutions were left to stand for 30 minutes. Absorbance reading of each of the sample was acquired using a UV Spectrophotometer and measurement was done at 517 nm. The antioxidant activity of each sample was calculated using the

formula:

where:

AC(0) = Absorbance of the blank at t = 0 mins

AS(t) = Absorbance of the sample at time t

Antimicrobial activity assay

Antimicrobial activity of crude and ethanol extracts from bignay was screened against common foodborne pathogens namely Escherichia coli and Staphylococcus aureus, and selected microorganisms that commonly cause spoilage in baked products namely Pseudomonas fluorescens, Bacillus subtilis, Candida albicans, and Aspergillus ochraceus. Pure cultures were obtained from the IFST Food Microbiology laboratory. Cultures were under refrigeration (4°C) as stock cultures and transferred weekly into fresh nutrient agar (NA) medium for bacteria, potato dextrose agar (PDA) for mold and malt yeast extract agar (MYA) for yeast to maintain viability. Pure cultures were used in the assay after 18-24 hours of incubation for bacteria and yeast and 32-48 hours for mold to make sure that the cells were in the stationary phase.

Susceptibility of the test organism to the extracts was determined by employing the standard paper disc diffusion technique (Proestos *et al.*, 2006). The microbial suspensions were diluted ten-fold in 0.85% NaCl solution and 0.1 ml from the appropriate dilution were spread plated on corresponding agar medium in order to give a population of approximately 106 cfu plate-1. Bignay berry crude extract and the alcoholic extract of different concentrations (25%, 50%, 75% and 100%) were prepared. Neutralized solutions were also prepared to test for the possible effect of pH on microbial inhibition. Appropriate amount of sodium carbonate was added to each of the extract in order to adjust the pH to 6.5. Sterile paper discs with a diameter of 12 mm were soaked into 50 µl of each extract and placed onto the inoculated agar surface. Petri dishes were incubated for 48 h at 37°C for E. coli, 30°C for S. aureus, B. subtilis and C. albicans, and for 72-96 h at 30°C for A. ochraceus. After incubation, the diameter of the zone of inhibition (in mm) was measured.

Determination of efficacy of bignay fruit extract as natural preservative in a baked product

Preparation of muffin

Muffin was prepared using the formulation by the Technology Resource Center of the Philippines (2007) with some modification. In a glass bowl, water, sugar and eggs were combined. Cooking oil, salt, baking soda, vanilla and lemon flavors and milk powder were added. Flour and baking soda were slowly added and the resulting batter was mixed thoroughly. Five treatments were prepared: control (without added preservative/antimicrobial agent), + 0.3% Ca propionate, + 5% bignay extract, + 10% bignay extract and + 15% bignay extract. The amounts of the bignay fruit extract added was based on the result of preliminary studies which consist of microbial and sensory evaluation.

Then, approximately 35 g of the mixture was poured into a greased muffin pan and placed in a pre-heated oven at 175°C for 20 min. After baking, the muffins were placed on a cooling rack until ambient temperature was reached. For each treatment, the sample was placed in a sterile polyethylene bag and was stored at ambient condition.

Microbial evaluation

The total microbial and mold counts of each sample were analyzed at 0, 2, 4 and 6 d of storage. Twenty five g of sample was mixed with 225 ml of 0.1% sterile buffered peptone water. The mixture was blended for 30 s in a laboratory stomacher. The mixture was serially diluted (1:10) with 0.1% of sterile peptone water and appropriate dilutions were pour plated in duplicate on Plate Count Agar (PCA) for the determination of total microbial count and Potato Dextrose Agar (PDA) for total mold count. PCA and PDA plates were incubated at 35°C for 24 hours and 30°C for 48 hours, respectively.

Sensory evaluation

Sensory evaluation of muffin was performed to determine if addition of bignay fruit extract has significant effect on specific attributes as compared with the control and the one with Ca propionate as antimicrobial. The intensity of different sensory attributes namely, color, aroma, flavor, and texture, as well as the general acceptability of the samples was determined by quality scoring and using a 15-cm line scale (Watts *et al*., 1989). These attributes were evaluated by 25 experienced panelists consisting of BS Food Technology students.

Statistical analysis

All experiments were carried out in triplicates. The experimental design used for the analyses was Completely Randomized Design (CRD). Randomized Complete Block Design (RCBD) was employed for the sensory evaluation. ANOVA was performed using the SAS General Linear Models (GLM) procedure with SAS software 9.1.3. Significance of differences was defined at $p \leq 0.05$. Differences

Table 1. Average zone of inhibition (mm) produced by the ethanolic extract from bignay fruit against the different test organisms (n=3)

Test microorganism	Ethanolic extract	Concentration (%)	Inhibition zone (mm)[a]
Escherichia coli	Red bignay	25	12.00 ± 0.00 b
		50	12.50 ± 0.50 ab
		75	12.83 ± 0.76 a
		100	13.00 ± 0.00 a
	Purple bignay	25	0.00 ± 0.00 c
		50	12.00 ± 0.00 b
		75	12.33 ± 0.58 ab
		100	12.83 ± 0.29 a
Staphylococcus aureus	Red bignay	25	12.00 ± 0.00 d
		50	12.67 ± 0.58 c
		75	13.17 ± 0.29 b
		100	13.67 ± 0.29 a
	Purple bignay	25	0.00 ± 0.00 e
		50	0.00 ± 0.00 e
		75	12.17 ± 0.29 d
		100	12.83 ± 0.76 b
Pseudomonas fluorescens	Red bignay	25	16. 50 ± 0.50 d
		50	17.83 ± 0.29 c
		75	19.67 ± 0.58 b
		100	25.00 ± 1.00 a
	Purple bignay	25	12.83 ± 0.76 f
		50	14.33 ± 0.58 e
		75	14.67 ± 0.58 e
		100	15.83 ± 0.76 d
Bacillus subtilis	Red bignay	25	15.83 ± 0.76 d
		50	17.67 ± 0.58 c
		75	23.17 ± 0.76 b
		100	25.17 ± 0.29 a
	Purple bignay	25	13.33 ± 0.58e
		50	15.33 ± 1.15 d
		75	16.00 ± 0.50 d
		100	16.17 ± 1.04 d
Candida albicans	Red bignay	25	12.00 ± 0.00 b
		50	12.83 ± 0.76 a
		75	13.00 ± 0.00 a
		100	13.17 ± 0.29 a
	Purple bignay	25	12.00 ± 0.00 b
		50	13.00 ± 0.00 a
		75	13.00 ± 0.00 a
		100	13.33 ± 0.58 a
Aspergillus ochraceus	Red bignay	25	0.00 ± 0.00 a
		50	0.00 ± 0.00 a
		75	0.00 ± 0.00 a
		100	0.00 ± 0.00 a
	Purple bignay	25	0.00 ± 0.00 a
		50	0.00 ± 0.00 a
		75	0.00 ± 0.00 a
		100	0.00 ± 0.00 a

[a]Means followed by the same letter (s) within the same species are not significantly ($p \leq 0.05$) different using LSD.

among treatments were examined for the level of significance using LSD.

Results and Discussion

Total phenolics and antioxidant activity of bignay fruit extract

The content of phenolic compounds in fruit is affected by the physiological maturity upon harvest, genetic differences (cultivar), pre-harvest environmental conditions, and post-harvest storage conditions and processing. Nevertheless, their concentration varies from plant to plant or even in different parts of the same plant at different ripening stage (Mahmood, 2012). Results of researches and other literature sources indicated that the variation in phytochemicals at physiological maturity depends mainly on the biosynthesis of the phytochemical during plant growth and development during physiological maturity (Tiwari *et al.*, 2011). Figure 1 shows the comparison of the phenolic content and antioxidant activity in terms of free radical scavenging activity of bignay fruits at two stages of maturity.

There is a marked increase in total phenol content (84.43 to 141.80 g CE 100 g^{-1}) of bignay fruits from the red mature (red) to the fully mature stage (purple). According to Mahmood and his colleagues (2012), there are two distinct phenomena of change in phenolic contents that were observed during

Table 2. Average zone of inhibition (mm) produced by the crude extract of bignay fruit against the different test organisms (n=3)

Test microorganism	Crude extract	Concentration (%)	Inhibition zone (mm)[a]
Escherichia coli	Red bignay	25	12.00 ± 0.00 b
		50	12.50 ± 0.50 ab
		75	12.83 ± 0.76 a
		100	13.00 ± 0.00 a
	Purple bignay	25	0.00 ± 0.00 c
		50	12.00 ± 0.00 b
		75	12.33 ± 0.58 ab
		100	12.83 ± 0.29 a
Staphylococcus aureus	Red bignay	25	0.00 ± 0.00 c
		50	12.33 ± 0.29 ab
		75	12.67 ± 0.58 ab
		100	12.83 ± 0.29 a
	Purple bignay	25	0.00 ± 0.00 c
		50	0.00 ± 0.00 c
		75	12.17 ±0.29 b
		100	12.50 ± 0.50 ab
Pseudomonas fluorescens	Red bignay	25	13.67 ± 0.29 d
		50	15.33 ± 0.58 c
		75	17.67 ± 0.58 b
		100	21.67 ± 2.08 a
	Purple bignay	25	0.00 ± 0.00 e
		50	12.67 ± 0.58 d
		75	13.67 ±0.58 d
		100	15.67 ± 0.58 c
Bacillus subtilis	Red bignay	25	15.50 ± 1.00 cd
		50	16.00 ± 1.00 cd
		75	21.33 ± 1.53 b
		100	23.83 ± 1.04 a
	Purple bignay	25	12.17 ± 0.29 f
		50	13.67 ± 0.58 ef
		75	14.50 ± 0.50 de
		100	16.17 ± 0.29 c
Candida albicans	Red bignay	25	12.00 ± 0.00 c
		50	12.50 ± 0.50 abc
		75	12.67 ± 0.58 ab
		100	12.83 ± 0.29 a
	Purple bignay	25	0.00 ± 0.00
		50	12.00 ± 0.00 c
		75	12.17 ± 0.29 bc
		100	12.50 ± 0.50 abc
Aspergillus ochraceus	Red bignay	25	0.00 ± 0.00 a
		50	0.00 ± 0.00 a
		75	0.00 ± 0.00 a
		100	0.00 ± 0.00 a
	Purple bignay	25	0.00 ± 0.00 a
		50	0.00 ± 0.00 a
		75	0.00 ± 0.00 a
		100	0.00 ± 0.00 a

[a]Means followed by the same letter (s) for the same species are not significantly ($p \leq 0.05$).different using LSD.

maturation: steady decrease or rise towards the end of maturation. Generally, for berries, higher phenolic and anthocyanin contents were reported in overripe fruit as compared with unripe ones (Tiwari *et al*., 2011).

Phenolics are compounds that can serve as antioxidants because of their redox property. This characteristic allows them to act as reducing agents, hydrogen donators, singlet oxygen quenchers, and radical scavengers (Proestos *et al*., 2006). As shown in Figure 1, extract of bignay fruit at the fully matured stage with greater amount of phenolics has higher antioxidant activity (87.10%) as compared with the less mature one (75.87%).

Antimicrobial activity of bignay fruit extract

Plants contain various constituents and are considered as valuable sources of biologically active molecules possessing antimicrobial properties. Plant extracts, either as standardized extracts or as a source of pure compounds, can be utilized for control of microbial growth owing to their chemical diversity (Negi, 2012). Recent researches have demonstrated that numerous berries high in bioactive phenolic chemicals have antimicrobial effects (Lacombe *et al*., 2012). Bignay contains relatively high amount of bioactive compounds like polyphenols that have detrimental effect on a wide range of microorganisms. In this study, the ethanol extract and the crude extract of the fruit were analyzed for antimicrobial activity

Table 3. Total count of muffin with different antimicrobial treatments at 0 to 6 days of storage (n=3)

Treatment	Total count (log CFU/ml)[a]			
	0 d	2 d	4 d	6 d
Control	1.37 ± 0.06 a	2.27 ± 0.03 a	4.12 ± 0.02 a	5.13 ± 0.02 a
+ 0.3% Ca propionate	1.12 ± 0.10 b	1.87 ± 0.08 b	2.57 ± 0.07 b	3.90 ± 0.02 b
+ 5 % bignay extract	- c	1.29 ± 0.11 c	1.58 ± 0.16 c	2.47 ± 0.03 c
+ 10 % bignay extract	- c	1.26 ± 0.07 c	1.52 ± 0.04 c	2.45 ± 0.05 c
+ 15 % bignay extract	- c	1.06 ± 0.10 d	1.49 ± 0.10 c	2.40 ± 0.08 c

- <1 log CFU/ml (below detection limit)
aMeans followed by the same letter (s) within a column are not significantly ($p \leq 0.05$) different.

Table 4. Mold count of muffin with different antimicrobial treatments at 0 to 6 days of storage (n=3)

Treatment	Mold count (log CFU/ml)[a]			
	0 d	2 d	4 d	6 d
Control	- a	- a	2.34 ± 0.03 a	3.09 ± 0.02 a
+ 0.3% Ca propionate	- a	- a	2.26 ± 0.02 a	3.02 ± 0.03 a
+ 5 % bignay extract	- a	- a	1.47 ± 0.07 b	2.09 ± 0.16 b
+ 10 % bignay extract	- a	- a	1.44 ± 0.13 b	2.08 ± 0.14 b
+ 15 % bignay extract	- a	- a	1.37 ± 0.06 b	1.96 ± 0.08 b

- <1 log CFU/ml (below detection limit)
aMeans followed by the same letter (s) within a column are not significantly ($p \leq 0.05$) different.

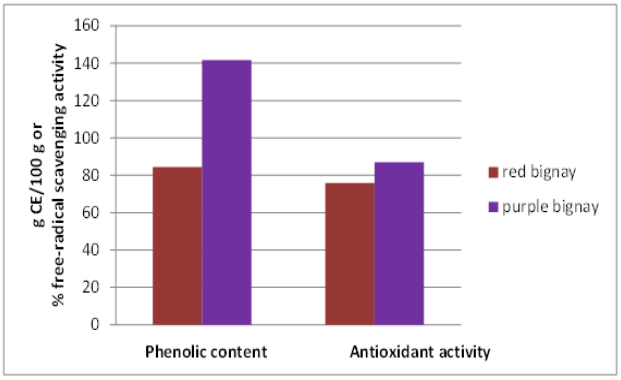

Figure 1. Comparison of the total phenolic content and antioxidant property of bignay at two stages of maturity

against pathogenic bacteria namely *Escherichia coli* and *Staphylococcus aureus*, and selected spoilage microorganisms namely *Pseudomonas fluorescens*, *Bacillus subtilis*, *Candida albicans*, and *Aspergillus ochraceus*.

Table 1 shows the antimicrobial effect of the ethanol extract of bignay against the different test organisms. Results show that ethanol extract from the red bignay fruit significantly produced the largest zones of inhibition. The extracts were effective against all bacterial strains used. Average diameter of the zones of inhibition produced by the extract against the bacteria *E. coli* and *S. aureus* ranges from 12.17-14.17 mm and 12.00-13.67 mm, respectively. The highest concentration, 100%, significantly produced the largest inhibition zone. The ethanol extract from purple bignay also produced zones of inhibition against the two pathogenic bacteria but these zones are relatively smaller than those produced by ethanol extract from red bignay.

The ethanolic extract from red bignay fruit also produced greater zones of inhibition against the spoilage bacteria *P. fluorescens* and *B. subtilis*. Average diameter of the zone of inhibition produced by the extract against *P. fluorescens* ranges from 16.50- 25.00 mm while the average zone of inhibition produced by the ethanolic extract from purple bignay ranges from 12.83-15.83 mm. The average zone of inhibition produced by ethanolic extract from red bignay against *B. subtilis* was significantly larger (15.83- 25.17 mm) as compared to that produced by the purple bignay ethanolic extract (13.33-16.17 mm).

On the other hand, ethanol extracts from both red and purple bignay also inhibited the growth of *C. albicans* and the inhibition zones produced at concentrations 50, 75 and 100% were not significantly different. However, the ethanol extracts were not proven to be effective against the mold *A. ochraceus*. The ethanolic extracts exhibited the greatest antimicrobial activity against the spoilage bacteria *P. fluorescens* and *B. subtilis*. Specifically, the ethanolic extracts derived from red bignay produced significantly larger inhibition zones against the two bacteria.

Similar observations have been noted upon the screening for the antimicrobial activity of the crude bignay extracts against the test organisms. The crude extract of red bignay was proven to be most effective against *P. fluorescens* and *B. subtilis*, with the average zones of inhibition ranging from 13.67- 21.67 mm and from 15.50-23.83 mm, respectively. Growth of other test microorganisms namely *E. coli*, *S. aureus*,

Table 5. Sensory scores of the muffin with different antimicrobial treatments (n=25)

TREATMENT	SENSORY ATTRIBUTES[a]				
	Color[b]	Aroma[c]	Texture[d]	Flavor[e]	General acceptability[f]
Control	8.67 a	7.33 a	8.63 a	5.93 a	9.20 b
+ 0.3% Ca propionate	7.94 a	7.05 a	8.22 a	6.62 a	10.20 b
+ 5 % bignay extract	5.20 b	7.45 a	5.64 c	7.38 a	13.26 a
+ 10 % bignay extract	5.80 b	8.12 a	6.44 bc	6.90 a	8.98 b
+ 15 % bignay extract	3.02 c	8.35 a	4.90 c	5.90 a	9.20 b

aMeans followed by the same letter (s) within a column are not significantly ($p \leq 0.05$) different using LSD.
[b]0=light brown, 15=dark brown
[c]0=weak, 15=strong
[d]0=soft, 15=tough/coarse
[e]0=weak, 15=strong
[f]0=unacceptable, 15=highly acceptable

and *C. albicans* were also inhibited but at a lower rate. The crude bignay extracts did not also show antimicrobial activity against *A. ochraceus*.

Table 2 shows the average zone of inhibition produced by the crude extracts from bignay against the different test organisms. According to Lacombe and colleagues (2012), mechanisms of berry phenolics' inhibition against bacteria have been proposed in recent studies. The outer bacterial membrane of bacteria provides the intrinsic barrier against chemicals. It is also supposed to be the primary target for berry phenolics. Cell walls of Gram-negative bacteria are composed of lipopolysaccharide (LPS), which provide inherent resistance against compounds that may cause damage to the cell. Anthocyanins and phenolic compounds can cause damage to the walls of these bacteria by destabilizing the LPS and increasing the efflux of ATP from the cytoplasm. This explains the significant antimicrobial effect of the bignay extracts against the Gram-negative bacteria *P. fluorescens* and considerable antimicrobial activity against *E. coli*. Gram positive bacteria possess thicker peptidoglycan cell walls which protect cells against hostile environments. Nevertheless, the bignay extracts also showed significant antimicrobial effect against the Gram-positive bacteria *B. subtilis*.

Exposure to low pH can cause sublethal injury to cell membranes, causing disruption of proton motive force owing to loss of Hþ-ATPase. This damage may make the bacteria more susceptible to the phenolic antimicrobial compounds. It was also hypothesized that phenolic compounds from some berries could bind the outer membrane of the cells, disrupting the permeability barrier of the outer membrane in Gram-negative bacteria. Variations in cell wall structures between Gram-positive and Gram-negative bacteria may cause different damage when the bacteria are subjected to antimicrobial compounds (Wu *et al.*, 2008).

Efficacy of bignay fruit extract as natural preservative in muffin

After screening for the antimicrobial activity of the different extracts, it was found that the extract from red bignay fruit exhibited the greater antimicrobial effect. Hence, it was the one used in food application. To determine the efficacy of bignay fruit extract as natural preservative in a baked product, muffin was prepared using the formulation by the Technology Resource Center of the Philippines (2007). Tables 3 and 4 show the total microbial count and total mold count for the different treatments at 0 d, 2 d, 4 d and 6 d storage period.

Results also show that the addition of at least 5% of bignay extract significantly inhibited the growth of microorganisms as compared with Ca propionate until the 6th day of storage at ambient condition. It was also observed that there was no significant difference among the treatments with varying concentrations of bignay extract in terms of the total microbial and mold counts. The use of natural antimicrobials in combination with another or with other technologies in a multi-hurdle preservation system, like in a baked product in which water activity is relatively low, can enhance the performance of natural antimicrobials (Negi, 2012).

Sensory evaluation

One of the main aspects that should be considered in the food application of natural antimicrobials is their possible effect on the sensory properties of foods (Taylor, 2012). In this study, sensory evaluation of muffin using quality scoring was performed in order to determine the effect of the use of bignay extract as natural preservative on the sensory attributes namely color, aroma, texture, and flavor, and the general acceptability of the product. Table 5 shows the evaluation of 25 panelists on the different sensory attributes of muffin.

The different treatments are not significantly different in terms of the baked aroma and flavor. In terms of color, results show that the muffin treated

with 15% red bignay extract had a significantly lighter color, followed by treatments with 5% and 10% extract, as compared with rest of the treatments. The slight lowering of pH upon addition of the extracts affected the development of brown color in the baked product. With the pH of the system influencing the ratio of browning products formed, the rate of color formation can be reduced by decreasing the pH (DeMan, 1999)

The different treatments also resulted in significant differences in the texture of the product. Muffins with bignay extracts were perceived to be softer than the control and the ones with 0.3% Ca propionate as preservative. Lastly, the sample with 5% bignay extract, in general, was considered by the 25 panelists to be the most acceptable among the treatments.

Conclusion

Based on the results, the bignay extracts from the red and fully mature fruits exhibit free radical scavenging antioxidant activity. Alcoholic and crude extracts from red bignay exhibited potent antimicrobial effects against *P. fluorescens* and *B. subtilis*. At 5% level, the extract of red bignay can significantly inhibit microbial growth in baked product like muffin. Addition of 5% extract also resulted in a softer and generally more acceptable muffin. Overall, bignay can be used as a natural ingredient with significant biological function due to its antioxidant and antimicrobial properties.

Acknowledgement

The authors would like to thank the Institute of Food Science and Technology, University of the Philippines Los Baños for providing the resources needed and making this study possible.

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