

The 3rd International Conference

of Multidisciplinary Approaches on UN Sustainable Development Goals (UNSDGs 2018)

December 28th – 29th, 2018

at the Hotel Windsor Suites & Convention, Bangkok, Thailand

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Proceedings

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Nakhon Pathom Rajabhat University

Table of Contents

Contents	Page
Plenary Abstracts	a
Presented Full Paper	b
Session of Sustainable Development Goals	1
Needs of using Google Docs in EFL classroom	2
Praxial interdisciplinary education and enquiry:Developing quality education at a Thai international college	5
Expanding roles of nursing in Taiwan SDGs	11
Session of Pure and Applied Science	17
Technology adoption and sustainable livelihood outcomes of farmers producing ethanol feedstocks in Thailand: A qualitative insight	18
Physicochemical properties, total phenolic content and antioxidant activities of aloe vera beverages	26
Attenuation coefficients of problock and red brick for gamma ray shielding applications	30
Radiation shielding properties for clay bricks and autoclaved aerated concrete bricks	33
Visible luminescence of Sm ³⁺ ions in Lithium Strontium Borate glasses	38
Session of Electrical Engineering and Computer Technology	43
The development of database system for alumni via web application	44
Risk assessment of Thai users disclosed Facebook's data privacy in each generation	49
Session of Medical Health Sciences and Laws	57
Efficacy of hair tissue based-therapy in male androgenic alopecia	58
The strategic movement in health science's curriculum toward the professional development view: A case study of Thai dental school	64
Sustainable development and intellectual property	67
An Insulin-Friendly Lifestyle for Optimal Health and the Prevention of Hyperinsulinemia, Metabolic Dysfunction, and Insulin Disease	70
Session of Nursing	78
Effectiveness of smoking cessation program applying the transtheoretical model among students of Siam University	79
Caring behavior of caregivers of elderly at Baan Bangkae social welfare development center	84

Contents	Page
The effect of Nei-Guan Acupressure on reducing postoperative nausea and vomiting in patients after surgery	91
Exploring the home experiences of parents caregiving for children with medical complexity: A qualitative synthesis	93
Exploring cancer patients' family caregivers views of cancer patients' choice of hospice	99
Development of the belief in treatment effectiveness scale for adults with chronic low back pain	101
Session of Humanities and Social Sciences	108
An IEP solution to the lessons learned from six years of online proficiency testing associated with the freshman and sophomore English courses in a technology university in Taiwan	109
English adjective order ability by L2 Thai learners	116
Do L2 experience, type of affix and motivational factors relate to affix knowledge in L2 English learning?	122
ESP course design in EFL classroom	127
Authentic assessment, What and why authentic assessment	131
Influencing factors for study master's degree of Nakhon Pathom Rajabhat University students	134
Difficulties in learning grammar of business English students at Nakhon Pathom Rajabhat University	138
Session of Environmental Engineering and Science	145
Impacts of climate change on irrigation water management by the Sirikit dam in Thailand	146
Gap analysis of environmental management system standard ISO14001:2015 conformity of a large school in Nakhon Pathom, Thailand	150
Participation and awareness of staff in the office that apply green offices standard under the principle of grounded theory	162
Environment management system assessment ISO14001:2015 of Honda car service center	167
Land snail as alternative food: safety and nutritional perspectives of Cyclophorus haughtoni	173
Session of Hospitality and Tourism Management	178
Potential ways creative health tourism activities in the Pathom Asoke community, Nakhon Pathom province	179
The development of Chiang Khan community identity for promoting tourism	183
The study of factors that affect making decision of agro-tourism at Klong Mahasawat Community, Putthamonthon District, Nakhon Prathom Provinc	187

Contents	Page
Guidelines for developing evaluation criteria of tourism trend indicators in capturing, tasting and sharing in Nakhon Pathom province	193
The study for a public relation media development guideline to promote the agro-tourism of Huai Muang communities, Kamphaeng Saen district, Nakhon Pathom	199
An approach for The quality development of historical sites in Wat Phra Pathom Chedi Ratcha Wora Maha Wihan, Muang District,Nakhon Pathom Province	205
The guideline of elderly tourist guides curriculum development for Koh Lad E-Tan subdistrict, Nakhon Pathom province	213
The comparative study of cultures between ASEAN and the plus three countries	217
Session of Buddhism for Thailand 4.0	221
The principles of social development in Theravada Buddhist philosophy	222
A study on problems and challenges faced by people due to Uma Oya water multi-purpose development project (UMDP) in Badulla district	226
Session of the Interdisciplinary Research	230
Non-English major undergraduate students' perception towards using English songs in foreign language classroom: A case of Thai–Nichi institute of technology	231
A study of EFL learners' satisfaction towards online learning	235
Mathematics teaching in basic education in Thailand by integrating STEM-waldorf technique to increase students' achievement and inspiration in learning	239
An analysis of the TOEIC test taking ability and needed skills for improvement of undergraduate students in Private University	247
The relationship of quality of work life among the staff at Photharam hospital	250
Factors affecting the loan pattern of member of savings cooperative of SCG Packaging Co., Ltd.	254

Physicochemical properties, total phenolic content and antioxidant activities of aloe vera beverages

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Abstract

The aim of this study was to investigate the physical and chemical properties, total phenolic content, and antioxidant activities of different proportions of Aloe vera juice (AVJ) that is used to manufacture Aloe vera beverages (AVB). Four AVB samples, called AVB1, AVB2, AVB3, and AVB4, were prepared by using different proportions of AVJ, grape juice concentrate (GJC), and sugar syrup (SS). These were made in ratios of 90:5:5, 90:0:10, 40:5:5, and 40:0:10 (% v/v/v), respectively. The results showed that pH and total soluble solid of the end products ranged from 4.64 to 4.75 and 9.05 to 11.12 oBrix, respectively. The different ratios of AVJ addition led to minimal changes in color. The AVB1 sample showed the highest total phenolic content (1.09 mg GA Eq./100 mL), followed by AVB2 (9.85 ± 0.41 mg GA Eq./100 mL). AVB1 and AVB2 showed the highest antioxidant activities as measured by metal chelating and the ABTS radical scavenging assay. The results, therefore, conclude that the addition of AVJ at 90% proportion showed significantly better improvements to the total phenolic content and antioxidant potential in AVB than 40% proportion.

Keyword: Aloe vera, beverage, total phenolic content, antioxidant activity

I. INTRODUCTION

Aloe vera (*Aloe barbasensis* Miller) is a perennial plant of Liliaceae family with turgid green leaves joined at the stem in a rosette pattern [1]. The gel of the leaves is associated with many polysaccharides. In fact, more than 200 bioactive chemicals have been found in A. vera gel which provide potentially positive effects on human health beyond basic nutrition [2]. The antioxidant compounds in A. vera may increase the stability and nutritional value of food [3]. Health benefits of A. vera include increasing high-density lipoprotein (HDL), reducing low-density lipoprotein (LDL) and blood sugar in diabetics, fighting acquired immune deficiency syndrome (AIDS), and improving the immune system.

The industries of A. vera products, such as beverage, dairy products, and food supplement, are very important to the economy and have been increasing year by year [4]. Additionally, A. vera has been widely utilized as a resource for functional food, especially healthy drinks contained A. vera gel [1]. Nowadays, Aloe juices with certain blends are very popular, for example lemon juice, sherbet, and electrolytes in sport drink, soluble fiber in a diet drink, vitamin B, amino acid and acetaminophen in a hangover drink, and vegetable juices in healthy drink. However, mucilaginous gel obtained from a fresh A. vera leave has a bitter taste and result in unpleasant taste sensations. The addition of some fruit juices is the easy technique to reduce the bitterness of the vegetable drink. However, the study of appropriate beverage formulation of A. vera gel blended with some fruit juices is

still lacking. Therefore, the aim of this work was to evaluate the effects of the different concentrations of A. vera juice (AVJ) on physical and chemical properties, total phenolic content, and antioxidant capacities in A. vera beverage (AVB).

II. MATERIALS AND METHODS

A. Preparation of Aloe vera juice

Fresh green and matured of A. vera leaves with uniform size were obtained from the Aloevera Herb International Co. Ltd., Bangkok. Grape juice concentrate (GJC), and sugar syrup (SS) were purchased from local store. The A. vera leaves were washed and kept in vertical position for about 1 h to facilitate the drainage of yellow liquid sap. The upper and lower rind portion was removed with the knife to separate the inner fillet portion, and further ground by blender and filtrated through the muslin cloth to obtained AVJ gel. Four different formulations of AVB containing different proportions of AVJ:GJC:SS (% v/v/v), namely AVB1 (90:5:5), AVB2 (90:0:10), AVB3 (40:5:5), and AVB4 (40:0:10) were prepared. The beverages were pasteurized at 85°C for 15 min, and then stored in green glass bottles at refrigeration temperature (4 ± 1°C) before analysis.

B. Physical and chemical analysis

The total soluble solids (TSS) was tested using a hand refractometer (HR-130, OPTIKA, Italy) and the pH value was

measured by pH meter (pHMaster LAB, Dynamica Scientific Ltd., UK). Instrumental color measurement was carried out using colorimeter (Chroma Meters CR-400, Konica Minolta, UK) calibrated with black and white standards. The color parameters of lightness (L^*), greenness/redness ($-/+ a^*$), blueness/yellowness ($-/+ b^*$), Chroma parameter (C) indicating color intensity, and hue angle (H°) were directly recorded for each sample. H° vary from 0° (pure red color), 90° (pure yellow color), 180° (pure green color), and 270° (pure blue color) [5].

C. Total phenolic content and antioxidant activities

The total phenolic content was estimated using a modified method of Follin-Ciocalteu method [6]. Briefly, 100 μL of AVB was dissolved in 2 μL of Na_2CO_3 followed by addition of 100 μL of Folin-Ciocalteu reagent. After allowing to stand for 30 min at room temperature, the mixture solution was measured the absorbance at 750 nm using UV-Vis spectrophotometer (Genesis 10 UV scanning, Thermo Fisher Scientific, USA). (The total phenolic content was expressed as mg gallic acid equivalent per 100 mL of AVB (mg GA Eq./100 mL sample).

ABTS radical scavenging assay (ABTS assay) was performed according to Wiriyaphan *et al.* [7]. $\text{ABTS}^{+\cdot}$ stock solution was prepared by mixing 7.4 mM of ABTS solution and 2.6 mM of potassium persulfate solution, in 10 mM phosphate buffer (pH 7.4), and kept in the dark for 16 h. Fresh $\text{ABTS}^{+\cdot}$ working solution was prepared by mixing $\text{ABTS}^{+\cdot}$ stock solution in 10 mM phosphate buffer (pH 7.4) (to attain the absorbance at 0.7 ± 0.02 , at 734 nm). Twenty μL of AVB sample was mixed with 1980 μL of $\text{ABTS}^{+\cdot}$ working solution, and then kept in the dark for 5 min before monitoring at 734 nm. Result was expressed as mg Trolox equivalents per 100 mL of AVB samples (mg Trolox Eq./100 mL sample).

Ferric reducing antioxidant power (FRAP assay) was carried out according to Wiriyaphan *et al.* [8] with slight modifications. Briefly, FRAP reagent was prepared by mixing 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ solution in 40 mM HCl, and 2.5 mL of 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution. 200 μL of AVB was mixed with 1 mL of fresh FRAP reagent and then incubated for 1h at room temperature before measuring the absorbance at 593 nm. The result was expressed as mg Trolox equivalent per 100 mL of AVB sample (mg Trolox Eq./100 mL sample).

Metal chelating assay was measured according to Decker and Welch [9] with slight modifications reported by Wiriyaphan *et al.* [9]. Briefly, 100 μL of AVB was mixed with 50 μL of 2 mM FeCl_2 and 100 μL of 5mM 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine). The mixture was incubated at room temperature in the dark for 20 min. The color of ferrous iron-ferrozine complex was monitored at 562 nm. Result was expressed as mg EDTA equivalents per 100 mL of AVB sample (mg EDTA Eq./100 mL sample).

D. Statistical analysis

The experiments were performed with arrangements in a completely randomized design (CRD) (and the mean value \pm standard deviations were presented. Data analyses were

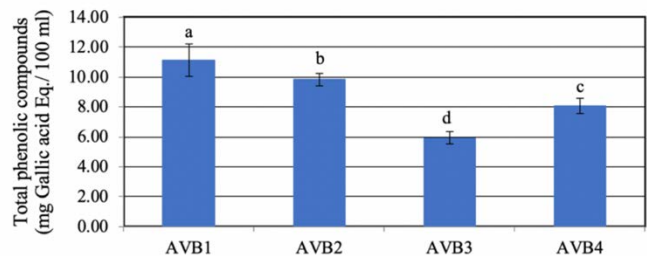
performed by ANOVA (analysis of variance) (and computed by the SPSS statistics for Windows, Version 17.0) SPSS Inc., Chicago, U.S.A. (Duncan's new multiple range test) DMRT (was used to determine significant differences among results and statistical significance was accepted at the 95 % probability) $p \leq 0.05$ (level).

III. RESULTS AND DISCUSSION

A. Physicochemical characteristic of Aloe vera beverage

The results obtained with respect to physical and chemical characteristics are presented in Table 1. Four blends of AVB with different proportions of AVJ, GJC, and SS caused little change of pH varied from 4.64 to 4.75. The TSS of AVB varied between 9.35 to 11.10 °Brix which was insignificantly affected by AVJ concentrations ($p > 0.05$).

For color characteristics were observed that L^* of 90% AVJ addition samples (AVB1 and AVB2, by 53.42 and 56.50, respectively) showed higher than 40% AVJ addition (AVB3 and AVB4, by 59.51 and 59.80, respectively), whereas b^* was observed with the higher value in AVB1 and AVB2, indicated that AVB with 90% AVJ addition exhibited more dark-yellow. While a^* showed negative value in all samples, indicating a slight green color. Comparable result was previously reported by Di Scala *et al.* [10], the initial colorimetric parameter L^* , a^* , and b^* of fresh A. vera gel were 52.00, -3.75, and 18.72, respectively. With regard to C and H° value, there were no significant differences in C and H° value of all samples. The samples presented the C and H° values ranging from 10.73 to 13.12 and 92.28 to 95.52, respectively, indicated that they had bright yellow color. In general, high H° value together with low C value is often indicative of a dull color [11].



Bars with differences letters indicate means with significant different ($p \leq 0.05$).

AVB1: 90% of Aloe vera juice, 5% of grape juice concentrate, and 5% of sugar syrup.

AVB2: 90% of Aloe vera juice and 10% of sugar syrup.

AVB3: 40% of Aloe vera juice, 5% of grape juice concentrate, and 5% of sugar syrup.

AVB4: 40% of Aloe vera juice and 10% of sugar syrup.

Fig. 1. Total phenolic content of each A. vera beverages (AVB).

B. Total phenolic content and antioxidant activities

Phenolics are considered as the main component of the plant, which can suppress free radicals [12]. The total phenolic content are shown in Fig. 1. It was found that AVB1 had the highest total phenolic content with the value 11.16 ± 1.09 mg GA Eq./100 mL, followed by AVB2 and AVB4, respectively, (9.85 ± 0.41 and 8.07 ± 0.52 mg GA Eq./100 mL, respectively). Comparable results were previously reported by Hulle *et al.* [13], who reported that the phenolic content for untreated and thermal treated of A. vera beverage mixed with

TABLE I. Physicochemical characteristic of each Aloe vera beverages (AVB).

Parameters	Values **			
	AVB1	AVB2	AVB3	AVB4
Biochemical attributes				
pH	4.68 ± 0.05 ^{a,b}	4.64 ± 0.06 ^a	4.75 ± 0.02 ^b	4.67 ± 0.08 ^a
TSS (^o Brix) ^{ns}	11.10 ± 0.85	10.12 ± 0.34	9.05 ± 3.05	10.35 ± 1.12
Color				
L*	53.42 ± 3.68 ^b	56.50 ± 1.80 ^{a,b}	59.51 ± 3.22 ^a	59.80 ± 4.70 ^a
a*	-1.10 ± 0.67 ^b	-0.60 ± 0.28 ^{a,b}	-0.72 ± 0.36 ^{a,b}	-0.48 ± 0.39 ^a
b*	11.92 ± 2.39	14.45 ± 1.82 ^a	10.69 ± 3.03 ^b	11.64 ± 0.92 ^b
Chroma (C) ^{ns}	^{a,b}	13.12 ± 0.90	10.73 ± 3.00	11.65 ± 0.90
Hue (H ^o) ^{ns}	11.99 ± 2.34	92.28 ± 1.71	94.54 ± 3.24	92.50 ± 2.05
	95.52 ± 4.00			

** : Values are expressed as mean ± S.D. (n = 6).

^{a-b}: Superscript letters with different letters in the same column indicate significant difference ($p \leq 0.05$).

ns: non-significant.

AVB1: 90% of Aloe vera juice, 5% of grape juice concentrate, and 5% of sugar syrup.

AVB2: 90% of Aloe vera juice and 10% of sugar syrup.

AVB3: 40% of Aloe vera juice, 5% of grape juice concentrate, and 5% of sugar syrup.

AVB4: 40% of Aloe vera juice and 10% of sugar syrup.

TABLE II. Antioxidant activity of A. vera beverages (AVB).

Sample	ABTS radical scavenging	FRAP value	Metal chelating activity
	activity (mg Trolox Eq./100 mL)	(mg Trolox Eq./100 mL) ^{ns}	(mg EDTA Eq./100 mL)
AVB1	3.08 ± 0.71 ^a	1.44 ± 0.18	5.61 ± 0.49 ^a
AVB2	3.34 ± 0.68 ^a	1.60 ± 0.42	4.75 ± 0.69 ^a
AVB3	1.41 ± 0.23 ^b	1.33 ± 0.27	2.27 ± 0.91 ^b
AVB4	3.23 ± 0.75 ^a	1.56 ± 0.05	2.26 ± 0.78 ^b

Values are expressed as mean ± S.D. (n = 6).

^{a-b}: Superscript letters with different letters in same row indicate significant difference ($p \leq 0.05$).

ns: non-significant.

AVB1: 90% of Aloe vera juice, 5% of grape juice concentrate, and 5% of sugar syrup.

AVB2: 90% of Aloe vera juice and 10% of sugar syrup.

AVB3: 40% of Aloe vera juice, 5% of grape juice concentrate, and 5% of sugar syrup.

AVB4: 40% of Aloe vera juice and 10% of sugar syrup.

litchi fruit was 33.6 and 42 mg GA Eq./100 mL, respectively. Whereas, total phenolic content of pure A. vera gel contained 37.70 mg GA E/q.100 mL, [10]. However, total phenolic content in AVB samples were lower than the previous studies, this might be due to the different of concentrations, thermal treatments and kinds of fruit juice blending as well as growth conditions of plant.

The antioxidant activities of plant extracts have been broadly used as the important parameter in order to evaluate their bioavailability as medicinal foodstuffs [12]. In this study, antioxidant activities were determined in accordance with three methods, including ABTS assay, FRAP assay, and metal chelating assay. The scavenging activity for the ABTS radical varied from 1.41 to 3.34 mg Trolox Eq./100 mL (Table 2). The samples of AVB1 and AVB2 showed the highest for

ABTS assay (3.08 ± 0.71 and 3.34 ± 0.68 mg Trolox Eq./100 mL, respectively), which showed the similar trend to metal chelating assay (5.61 ± 0.49 and 4.75 ± 0.69 mg EDTA Eq./100 mL, respectively). Although, antioxidant activity evaluated by FRAP assay was not significantly ($p > 0.05$) different between each sample. These results indicated that high concentration of AVJ addition impacted on greater antioxidant activities. The sample with high concentration of AVJ had greater amount of phenolic compound that highly correlated with antioxidant activity. The ability of phenolic compound involves antioxidant activity with different mechanism, including scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations [14]. Heş *et al.* [15] confirmed the metal chelating ability of aloe extract. Moreover, Nejatzadeh-Barandoz [16] noticed the ability of A. vera gel extract on ferric ion reducing powder. In generally,

phenolic compounds can play an important role on reducing power activity which is based on chelation of Fe^{2+} ions in a quantitative manner by the reagent ferrozine, resulting in the formation of a complex with Fe^{2+} ions. Therefore, the chelating ability influences other scavenging activities of free radicals which protect the organisms against oxidative damage [17, 18]. Nevertheless, the concentrations of phenolic compounds are not the only factor influencing antioxidant capacity, but their structural arrangements (number and position of hydroxyl groups, double bonds, and aromatic rings) also play the key role [19].

V. CONCLUSION

Based on the results, the concentration of AVJ leads to minimal changes in physical and chemical properties, including pH, TSS, and color of the AVB. The AVB samples with 90% proportion of AVJ addition promoted a significant increase in antioxidant activities by metal chelating agent assay and ABTS assay, and total phenolic content, but did not affect to FRAP assay value. Consequently, it can be concluded that the AVB samples with 90% AVJ addition was the suitable condition of healthy beverage. However, further clinical trials regarding these claims are necessary before accurate conclusions regarding these health benefits can be made

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