ปริมาณสารประกอบฟีนอลทั้งหมดและปริมาณแกลลิกในผลมะขามป้อมสดและผลมะขามป้อมดองจากห้าแหล่งสายพันธุ์

TOTAL PHENOLIC AND GALLIC ACID CONTENTS IN FRESH AND PRESERVED *EMBLICA OFFICINALIS* GAERTN FRUITS FROM FIVE DIFFERENT VARIETIES

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บทคัดย่อ

ประเทศแถบเอเชียมีการใช้มะขามป้อมในทางยารักษาโรคหลายชนิดในยาแผนโบราณ ในประเทศไทยใช้เพื่อบำรุงสุขภาพและเป็นหนึ่งในตำรับยาอายุรเวทที่มีรายงานในการต้านมะเร็ง กรดแกลลิคเป็นสารสำคัญที่มีสรรพคุณต้านการเกิดออกซิเดชั่นที่ใช้แพร่หลายในอุตสาหกรรมเภสัช ดังนั้นงานศึกษานี้วัตถุประสงค์เพื่อวิเคราะห์ปริมาณกรดแกลลิกและสารประกอบฟีนอลทั้งหมด ในผลมะขามป้อมสดและดองด้วยสารละลายเกลือ 10% และ 15% จากห้าสายพันธุ์โดยมีระยะการเก็บรักษา สามเดือน มะขามป้อมห้าสายพันธุ์ ได้แก่ สายพันธุ์ KR-1, KR-2, KR-3, KR-4 and LTK-3 วิธีการวิจัยเริ่มจากการแกะเนื้อออกจากเมล็ดมะขามป้อม อบแห้งและบดละเอียดตามลำดับ จากนั้นสกัดผงมะขามป้อมโดยใช้เอธานอลที่มีความบริสุทธิ์สูงและวิเคราะห์ปริมาณกรดแกลลิกและสารประกอบฟีนอลทั้งหมดในสารสกัดด้วยเครื่องมือ HPLC ผลการวิเคราะห์พบว่าปริมาณกรดแกลลิกน้อยที่สุดในสายพันธุ์ KR-4 และมากที่สุดในสายพันธุ์ LTK-3 นอกจากนี้ปริมาณสารประกอบฟีนอลทั้งหมดเพิ่มขึ้นจากสัปดาห์ที่ 0 ถึงสัปดาห์ที่ 12 การวิจัยจะดำเนินการในทางด้านผลิตภัณฑ์อาหาร การศึกษาฤทธิ์ทางยาและเครื่องสำอางจากมะขามป้อมสายพันธุ์ LTK-3 ต่อไป

คำสำคัญ: มะขามป้อม วงศ์ยูฟอร์เบียซี กรดแกลลิก สารประกอบฟีนอลทั้งหมด การวิเคราะห์ด้วยเครื่องมือเฮชพีแอลซี

Abstract

Emblica officinalis Gaertn has been used in traditional medicine for treatment of various diseases in Asian countries. It has been recorded in Ayurvedic and traditional medicines in Thailand for health tonic and one of its recipe was recently reported on cancer treatment. Gallic acid, an active compound from *E. officinalis* fruit varaints, has revealed on research against oxidative diseases and used in pharmacological manufacturing. This study was aimed to analyze gallic acid and total phenolic phytoconstituents in *E. officinalis* Gaertn fruits from five difference varieties in both fresh and their preserved in 10% and 15% NaCl solution during three months storage time. The five fruit pulps from KR-1, KR-2, KR-3, KR-4 and LTK-3 were separated from the seeds, then have been dried and powdered, respectively. The powder was extracted by using absolute ethanol. Gallic acid content was evaluated in all extracts by using HPLC apparatus. The results revealed that KR-4 variety contained the lowest gallic acid content whereas the highest content was obtained in LTK-3 variety. Gallic acid and phenolic contents were increased from week 0 to 12. The further research will focus on food products, pharmaceutical evaluation and cosmetics in the LTK-3 variety.

Keywords: *Emblica Officinalis* Gaertn, Euphorbiaceae Family, Gallic Acid, Total Phenolic Content, HPLC Analysis.

Introduction

Emblica or Indian gooseberry (*Emblica officinalis* Gaertn syn. *Phyllanthus emblica* Linn.), called in Thai as Ma-Kham-Pom; Amla in Hindi, An Mole in Chinese, Neli in Tamil, belongs to the family Euphorbiaceae. Jan, *et al*. [1] Five fruits variants of *E. officinalis* have used in this experiment (Fig. 1). It is one of most important medicinal plants have used in traditional systems and treated of various diseases. Maurya, *et al*. [2]. This fruit has evidence been used in Ayurveda as diarrhea, jaundice, anti-inflammation ailments. In Thai traditional and Ayurvedic medicines, "Triphala" formulas mixture of three plant fruits including *E. officinalis*, *Terminalia chebula* Retz and *Terminalia bellerica* (Gaertn.) has been used as health tonic and were recently reported for cancer treatment. Wongnoppavich, *et al*. [3] The pharmacological research has reported on analgesic, antitussive, antiatherogenic, adaptogenic, neuroprotective, anticancer, chemopreventive and other properties, Srivasuki, *et al*. [4] and Zhang, *et al*. [5] have reported that the fruit is highly nutritious food rich of vitamin C, amino acids and minerals, containing several chemical constituents such as tannins, alkaloids and phenols. Hydrolysable tannins such as emblicanin A, B, gallic acid and ellagic acid, are reported to possess anti-oxidant activity, Singh, *et al*. [6]. Sawant, *et al*. [7], has studied gallic acid and ascorbic acid can be used as chemical markers for quality control and standardization of this plant and its formulation. HPLC method represented an excellent technique for

simultaneous determination of gallic acid and ascorbic acids in the extract of *E. officinalis*, Sawant, *et al*. [7]. Rakesh, *et al*. [8] has reported that the fruit was high acidity and astringency according to those constituents; therefore, it was commonly processed into various value-added products such as candy, juice, pickle, powder, segments-in-syrups, etc. During the process and storage, the juice suffers from severe browning and loss of ascorbic acid during storage at room temperature. Bhattacherjee, *et al*. [9]. The quality of fresh fruit and the preservation related to gallic acid during storage is in our interesting remark. Phenolic compounds are widely spread throughout the plant kingdom and have potential against oxidative damages diseases. Gallic acid is a type of phenolic compounds and common used in the pharmaceutical industrials. It is used as a standard for determining the phenolic content of various analysts, for example Folin-Ciocalteau assay. Rurnroengklin, *et al*. [10] indicated that the temperature of 45-60^{0}C or pH 3-4 exhibited a relative high antioxidant activity and helped to improve extraction yield of dietary phenolics Reblova, *et al*. [11] reported that the antioxidant activity of phenolics, including gallic acid showed a slower decrease in antioxidant activity with increasing temperature compared to the activity at 90^{0}C. Gallic acid, protocatechuic and caffeic acids exhibited a significant antioxidant activity at 150^{0}C whereas vanilic acid was active only at 90^{0}C. Moldovan, *et al*. [12] illustrated food preservatives used in aqueous were found to have a slightly influence on the anthocyanin stability. Therefore, this study was aimed to determine for gallic acid content (Fig. 2C) in five variants of fresh fruits and their preserved in 10% (w/v) and 15% (w/v) aqueous sodium chloride which have been stored in cold room at 4^{0}C for 12 weeks.

Figure 1. Fruit variants of *Emblica officinalis* Gaertn used in this study

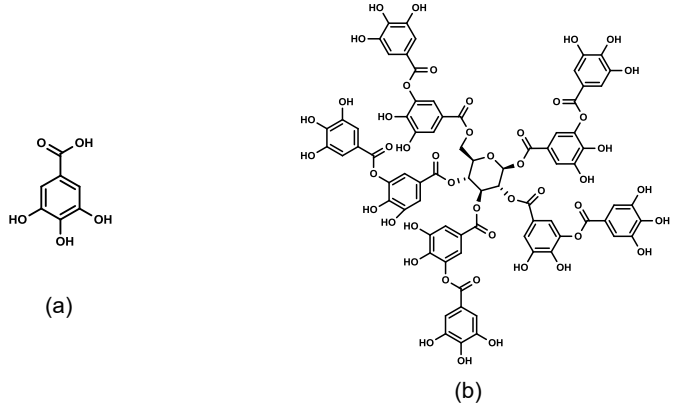

Figure 2. Chemical structure of gallic acid (a), and tannin (b)

Objectives

Purpose of this study was to analyze gallic acid and total phenolic contents, constituent in *E. officinalis* Gaertn fruits pulps from five difference varieties such as KR-1, KR-2, KR-3, KR-4 and LTK-3., grown in the same area. The evaluation was compared between the fresh fruits and the preserved in 10% and 15% aqueous NaCl solution during three months, temperature 4^{0}C in cold room storage.

Methods

1. Plant materials

The fresh fruit of *E. officinalis* from different varieties, including KR-1, KR-2, KR-3, KR-4 and LTK-3 (Table 1) were collected by Ms. Cholticha Niwaspragrit and Mr. Maitree Munyanont from Lam Ta Kong Research Station, Thailand Institute of Scientific and Technological Research (TISTR), Nakhonratchasima province, Thailand. Post-harvest after collected the fruits from their trees, put them into plastic bags, then carried them to the Agricultural department, TISTR, Pathumthanee by car for 3 hrs. They were stored in 12-13^{0}C before using in this experiment.

Table 1 Plant materials detail has used in this experiment.

Varieties	Post-harvest After Flowering (days)	Fresh Fruit Diameter (cm)	Fruit Height (cm)	Fresh Fruit Weight (g)
KR1	180	3.34	2.80	18.19
KR2	180	3.66	3.12	24.12
KR3	180	3.63	2.97	23.10
KR4	180	3.12	2.76	15.34
LTK3	180	2.49	2.40	8.64

2. Instruments

Rotary evaporator; Rotavapor R-3, Vacuum pump, V-700, Buchi, Switzerland, Cooler and pump VTL 911, Shinho.; Oven: Kluay Nam Thai Oven, Bangkok, Thailand. Cold storage room, temperature control at 4^{0}C, Riva Cold Service Limited, Bangkok, Thailand.; Blender; Blenforce glass 500W, Tefal, France, supplied in Thailand.; HPLC; 20AD Prominence, Shimadzu, Japan, Photodiode Array Detector (PDA).

3. Chemicals

Ethanol; AR grade, AR1069-G4L, RCI Labscan Asia, supplied in Thailand; Methanol; HPLC grade, RCI Labscan Limited, Asia, supplied in Thailand. HPLC standard (Gallic acid); CAS-149-91-7, code: 410860050, purity 98%, ACROS, New Jersey, USA.; Acetonitrile; HPLC grade, RCI Labscan Limited, supplied in Thailand.; Citric acid monohydrate; BP98 8-80 mesh, MO11754, TTCA CO., LTD. Shandong, China; Potassium

Metabisulfide; Food grade (E224), BASF SE, Ludwigshafen, Germany.

4. Extraction of Gallic Acid

The *E. officinalis* fruits were washed and pickled in 10% (w/v) and 15% (w/v) sodium chloride solution (NaCl aq.), containing 0.5% (w/v) citric acid and 500 ppm. potassium metabisulfite. The fresh and preserved fruits were kept in cold room (4^0C) for 12 weeks and were randomly collected in weeks 0, 2, 4, 6, 8, 10 and 12, respectively. The fruit samples were, washed, removed the seed, dried in oven (50^0C) for 21 hours, and grinded. The powder with 4.88% moisture was extracted with a powder/ethanol ratio (1:3w/v) at room temperature, pH 4.04 for 15 days [13] with occasionally shaking. The extract was evaporated in vacuum by using rotary evaporator (45^0C, 180-190 mbar) and 4.88% moisture of dried powder. The gallic acid content was determined from dry extracts by using high performance liquid chromatography (HPLC).

5. Sample Preparation for HPLC

The accurately weighed (0.1030 g) of *E. officinalis* extractions were transferred in 50 mL volumetric flask and sonicated for 20 min at 27 ± 3^0C in sonicator water bath. The solution, pH 6, was filtered through a 0.45 µm membrane prior to injection into the HPLC.

6. Determination of Gallic Acid Content by HPLC

The HPLC analysis method has followed from Nasr, *et al.* [14]; Govindarajan, *et al.* [15]; Sawant, *et al.* [7], the samples were triplicated in the experiment and the quantitative analysis were calculated from the equation as well as HPLC condition have shown below. HPLC; 20AD Prominence, Shimadzu, Japan, Photodiode Array Detector (PDA). Injection volume: 20µL Column: Ultra BiPh 5 um, 250x4.6 mm. Flow rate: 0.8 mL/min. (Gradient) Mobile phase: A = 0.3% phosphoric acid, B = acetonitrile: H_2O: phosphoric acid (79.7:20:0.3). Fit type: Linear, Retention time 7.105, detection on peak area. Standard: gallic acid, purity 98%, ACROS.

Calculation Equation

Y = mX+b or mCo+b

X = y-b/m = Co

Gallic Acid (mg/Kg) = Co x V x DF/Wt. (g) x 1000

A. Parameters

Y = Chromatogram Area/Height

m = Slope of Linear Line

X = Co = Standard Concentration (mg/mL)

b = Standard Curve Intersection, Wt. = Sample Weight (g)

Results

Table 2 Plant materials detail has used in this experiment.

Varieties	Fresh Fruit Weight (g)	Fresh Pulp Weight (g)	Dried Pulp Weight (g)	% Moisture of Powder
KR1	18.19	15.94	2.79	82.50
KR2	24.12	21.43	2.59	87.91
KR3	23.10	20.83	2.51	87.95
KR4	15.34	13.32	1.97	85.21
LTK3	8.64	7.63	1.70	77.33

Table 3 Total phenolic content extracted from fresh and preserved *E. officialis* fruits at different storage times.

Varieties	Storage at 4°C	Total phenolic content (mg GAE/mg ethanolic extract of *E. officinalis* fruit)*						
		Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
KR1	fresh	0.22 ± 0.01^{1aA}	0.20 ± 0.01^{1aA}	0.20 ± 0.01^{1aA}	nd	0.13 ± 0.00^{2aA}	nd	0.06 ± 0.00^{1A}
	10% NaCl	0.09 ± 0.01^{1aA}	0.17 ± 0.01^{1aA}	0.20 ± 0.01^{1aA}	0.20 ± 0.00^{1aA}	0.18 ± 0.00^{2aA}	0.20 ± 0.01^{1aA}	nd
	15% NaCl	0.19 ± 0.01^{1aA}	0.18 ± 0.01^{1aA}	0.18 ± 0.00^{1aA}	0.13 ± 0.00^{1aA}	0.20 ± 0.01^{2aA}	0.18 ± 0.01^{1aA}	nd
KR2	fresh	0.22 ± 0.01^{1aA}	0.24 ± 0.02^{1aA}	0.24 ± 0.00^{1aA}	nd	0.22 ± 0.01^{12aA}	nd	0.17 ± 0.00^{1A}
	10% NaCl	0.18 ± 0.01^{1aA}	0.14 ± 0.01^{1aA}	0.21 ± 0.00^{1aA}	0.17 ± 0.00^{1aA}	0.18 ± 0.01^{12aA}	0.18 ± 0.01^{1aA}	nd
	15% NaCl	0.15 ± 0.01^{1aA}	0.17 ± 0.00^{1aA}	0.18 ± 0.01^{1aA}	0.19 ± 0.00^{1aA}	0.18 ± 0.00^{12aA}	0.18 ± 0.00^{1aA}	nd
KR3	fresh	0.23 ± 0.01^{1aA}	0.25 ± 0.01^{1aA}	0.25 ± 0.01^{1aA}	nd	0.19 ± 0.00^{12aA}	nd	0.16 ± 0.00^{1A}
	10% NaCl	0.18 ± 0.01^{1aA}	0.16 ± 0.01^{1aA}	0.21 ± 0.01^{1aA}	0.19 ± 0.01^{1aA}	0.21 ± 0.00^{12aA}	0.22 ± 0.00^{1aA}	nd
	15% NaCl	0.19 ± 0.01^{1aA}	0.18 ± 0.01^{1aA}	0.18 ± 0.01^{1aA}	0.18 ± 0.01^{1aA}	0.17 ± 0.01^{12aA}	0.18 ± 0.00^{1aA}	nd
KR4	fresh	0.12 ± 0.01^{2aA}	0.12 ± 0.01^{2aA}	0.08 ± 0.00^{2aA}	nd	0.07 ± 0.00^{3aA}	nd	0.04 ± 0.00^{1A}
	10% NaCl	0.08 ± 0.01^{2aA}	0.08 ± 0.01^{2aA}	0.09 ± 0.00^{2aA}	0.09 ± 0.01^{2aA}	0.10 ± 0.00^{3aA}	0.09 ± 0.00^{2aA}	nd
	15% NaCl	0.08 ± 0.01^{2aA}	0.08 ± 0.01^{2aA}	0.09 ± 0.00^{2aA}	0.09 ± 0.00^{2aA}	0.08 ± 0.00^{3aA}	0.09 ± 0.00^{2aA}	nd
LTK3	fresh	0.25 ± 0.01^{1aA}	0.24 ± 0.01^{1aA}	0.25 ± 0.01^{1aA}	nd	0.20 ± 0.01^{1aA}	nd	0.20 ± 0.00^{1A}
	10% NaCl	0.23 ± 0.01^{1aA}	0.19 ± 0.00^{1aA}	0.22 ± 0.00^{1aA}	0.22 ± 0.01^{1aA}	0.24 ± 0.01^{1aA}	0.20 ± 0.01^{1aA}	nd
	15% NaCl	0.21 ± 0.01^{1aA}	0.21 ± 0.01^{1aA}	0.23 ± 0.00^{1aA}	0.21 ± 0.01^{1aA}	0.23 ± 0.01^{1aA}	0.20 ± 0.01^{1aA}	nd

* Each value is expressed as the mean ± S.D.

The different letters within the same column represent significantly different in the values ($P<0.05$).

Table 4 Quantitative analytical data of Gallic acid (mg/kg) content extracted from fresh and the preserved *E. officialis* fruits at different storage times.

Fruit Varieties	Gallic Acid (mg/kg) Extracted From *E. Officialis* Fruits						
	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
KR-1 fresh	6.33 ± 1.01^{bA}	4.72 ± 0.32^{cA}	12.82 ± 2.13^{deB}	nd	15.72 ± 0.64^{eC}	nd	5.01 ± 0.08^{bA}
KR-1 10%NaCl	6.29 ± 0.11^{bA}	7.66 ± 0.82^{dA}	7.21 ± 2.48^{bA}	5.35 ± 0.44^{bA}	7.57 ± 0.43^{cA}	6.95 ± 0.45^{bA}	nd
KR-1 15%NaCl	12.57 ± 0.08^{eC}	8.63 ± 0.84^{dB}	6.12 ± 0.16^{bA}	5.17 ± 0.84^{bA}	12.54 ± 0.97^{dC}	7.66 ± 0.62^{bB}	nd
KR-2 fresh	9.74 ± 0.11^{dA}	8.25 ± 0.19^{dA}	10.16 ± 1.55^{cA}	nd	32.73 ± 0.29^{iC}	nd	26.28 ± 0.22^{cB}
KR-2 10%NaCl	16.01 ± 0.27^{fB}	12.41 ± 0.73^{eB}	11.37 ± 0.37^{cdA}	12.71 ± 0.86^{deB}	20.48 ± 0.63^{gD}	15.34 ± 1.01^{dC}	nd
KR-2 15%NaCl	20.36 ± 0.13^{hB}	12.59 ± 0.70^{eA}	11.71 ± 0.68^{cdeA}	13.69 ± 1.07^{eA}	20.53 ± 0.70^{gB}	16.66 ± 2.20^{dB}	nd
KR-3 fresh	7.25 ± 0.07^{cA}	21.70 ± 1.42^{gD}	16.77 ± 0.94^{fC}	nd	17.38 ± 0.29^{fC}	nd	11.52 ± 0.14^{bB}
KR-3 10%NaCl	18.27 ± 0.10^{gD}	8.47 ± 0.44^{dA}	11.70 ± 0.67^{cdeB}	11.59 ± 0.42^{dB}	14.78 ± 0.31^{eC}	12.03 ± 1.34^{cB}	nd
KR-3 15%NaCl	18.60 ± 0.64^{gC}	8.39 ± 0.72^{dA}	10.32 ± 0.79^{cB}	10.22 ± 0.96^{cB}	7.24 ± 0.16^{cA}	11.12 ± 0.27^{cB}	nd
KR-4 fresh	2.39 ± 0.81^{aB}	1.11 ± 0.11^{aA}	2.32 ± 0.27^{aB}	nd	3.42 ± 0.18^{aC}	nd	4.99 ± 0.12^{aD}
KR-4 10%NaCl	6.89 ± 0.24^{bC}	2.60 ± 0.01^{bA}	3.20 ± 0.35^{aAB}	2.19 ± 0.14^{aA}	3.81 ± 0.08^{aB}	4.10 ± 0.05^{aB}	nd
KR-4 15%NaCl	6.56 ± 0.26^{bE}	2.71 ± 0.16^{bB}	3.34 ± 0.33^{aB}	1.89 ± 0.46^{aA}	5.02 ± 0.07^{bcD}	4.57 ± 0.34^{aD}	nd
LTK-3 fresh	2.43 ± 0.71^{aA}	8.28 ± 0.19^{dB}	18.20 ± 1.13^{fC}	nd	38.64 ± 0.19^{kD}	nd	74.30 ± 0.82^{dE}
LTK-3 10%NaCl	27.74 ± 0.72^{iD}	12.47 ± 0.11^{eB}	9.95 ± 0.06^{cA}	12.44 ± 1.06^{deAB}	26.37 ± 1.59^{hD}	14.82 ± 0.47^{dB}	nd
LTK-3 15%NaCl	25.63 ± 0.59^{iC}	13.12 ± 0.45^{fAB}	13.83 ± 0.84^{eAB}	13.61 ± 0.22^{eA}	25.80 ± 0.91^{hC}	15.08 ± 1.28^{dB}	nd

*Each value is expressed as the mean $\pm$S.D.

The different letters within the same column represent significantly different in the values ($P<0.05$).

Conclusions and Discussion

From the data provided in Table4, revealed that KR-4 found the lowest quantity of gallic acid in both obtained from preserved fruit in 10% and 15% NaCl solution during the 10 weeks while the higher content was exhibited from LTK-3, and followed by KR-2, KR-3 and KR-4, respectively. The increase of gallic acid content was found between the extracts from week0 and week 10 for all sample tested. The pickled fruits exhibited higher gallic acid content than that of fresh fruits, especially which preserved in 15% NaCl solution. Moreover, the stability evaluation of CAPROS® containing *E. officinalis* and the sample was kept at ambient temperature for 24 months illustrated that gallic acid was increased in the percentage by twice in the first year during 12 months. The report showed gallic acid content was increased as storage time increased [16]. The tannin quantity was decreased from the week 0

compared to week 12 in the same plant material in reported previously. Gallic acid was found in the opposite direction to tannin content but it was illustrated in the similar way compared to phenolic content in this study. The evaluated the total phenolic content derived from the plant extracts as the present experiment was increased during the 12 weeks' period (Table 3) which was related to gallic acid content obtained from the present study. Gallic acid is mainly produced by hydrolysis of tannic acid, a specific form of tannin obtained from Chinese gall and the starting material for hydrolysable tannin synthesis [17-18]. Moreover, gallic acid is a precursor for the antimalarial drug trimethoprim and food preservative which derived from tannin hydrolysis [19-20]. Shete, *et al*. [21] reported a two-step bioconversion of natural tannin rich source into gallic acid by using tannase enzyme. Furthermore, Bourgou, S. *et al*. [22] studied the effect of Nacl on fatty acids, phenolics and oxidant activity of *Nigella sativa* organ revealed that salt stress increased phenolic content in shoots but decreased that in roots. The influence of salinity on phenolic acid production was involved to plant biosynthesis. The low salinity favoured the biosynthesis of major hydroxybenzoic compound, vanillic acid, while at higher NaCl doses; carbon was used for the phenolic acid groups including benzoates and cinnamate, especially trans-cinnamic acid, precursor of different hydroxyl-cinnamates. Whereas the production of *p*-hydroxybenzoic (gallic acid), syringic and chlorogenic acids in low and moderate salinities. In this study, gallic acid content was possibly increased from tannin decomposition from the increasing concentration of NaCl. Salinity is a major a biotic affecting plant grow, secondary metabolism. The anti-oxidation capacity of phenolic and flavonoids found to increase at low (25 mM) and moderate (50 mM) levels but were declined at severe (75 mM and 100 mM) levels [23].

It was interested that gallic acid content was increased during the period of time and the picked fruits contained higher gallic acid content than the fresh one. The results also showed that pickled fruits in 15% NaCl solution illustrated a higher gallic content than those in 10% NaCl solution. Gallic acid is useful as functional food, and pharmaceutical products, therefore, the future research will focus on development of food and pharmacetical products from *E. officinalis*, especially LTK-3 fruit variety, according to the highest gallic acid content.

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