ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Morphology, Thermal and Antioxidative Properties of Water Extracts from *Sonneratia caseolaris* (L.) Engl. Prepared with Freeze Drying and Spray Drying

Thawatchai Phaechamud[1]*, Kotchamon Yodkhum[1], Chutima Limmatvapirat[2] and Penpan Wetwitayaklung[3]

[1]Department of Pharmaceutical Technology, [2] Department of Pharmaceutical Chemistry, [3]Department of Pharmacognocy, Faculty of Pharmaceutical, Silpakorn University, Nakorn Pathom, 73000, Thailand.

ABSTRACT

Phenolic compound, such as gallic acid, and two flavonoids, e.g. luteolin and luteolin-7-O-glucoside, are the interesting substances in cork tree (*Sonneratia caseolaris* (L.) Engl.). Utilization of cork tree has been reported as traditional medicine and food. Generally, herbal plants are employed as fresh plant or drying and processing into dietary supplement. Therefore in this study, extracts from several parts of cork tree were prepared using boiled water to emulate the usage. The dried extracts were obtained using two drying techniques, spray drying and freeze drying. Morphology and thermal properties of the dried extracts were investigated using scanning electron microscope (SEM) and differential scanning calorimetry (DSC), respectively. Total phenolic compound amount was determined using Follin-Ciocalteu technique. The antioxidant activity of the extracts were measured using TEAC (Trolox Equivalent Antioxidant Capacity) and FRAP (Ferric reducing antioxidant power) methods. Squeezed juice from the fresh plant was also analyzed for the comparison. Morphology of the spray-dried extracts powder was spherical shape with highly shrinkage. The difference was observed for the leaf extract that found less shrinkage than the others. Two endothermic peaks around 60°C and 150-160°C were evident in DSC thermogram of almost exracts whereas that of leaf extract was found around 130-140°C. The highest % yield of the water extract was obtained from stamen following by calyx and leaf of cork tree. Total phenolic compound containing in the extracts was found lower than that of the fresh plant. Thermal drying process reduced the amount of total phenolic compound. Antioxidant activities of the extracts from stamen, calyx and leaf reported in terms of TEAC value were in medium range comparing to the standard antioxidant, trolox. GEAC (Gallic acid Equivalent Antioxidant Capacity) value from FRAP method was rather low comparing to gallic acid. However the rather high total phenolic compound and antioxidant, the cork tree exhibited the ability as the source of dietary supplement.

KeywordS: *Sonneratia casseolaris* (L.) Engl., water extract, spray dry, freeze dry, antioxidant

Corresponding author
Email:thawatchaienator@gmail.com

ISSN: 0975-8585

INTRODUCTION

Cork tree (*Sonneratia caseolaris* (L.) Engl., family Sonneratiaceae) is one of the lead plants of the mangrove forest. Fire-flies reside in this tree and emit light around during the night. Some utilization of cork tree are such as plugging its fruit with a stick and playing it as a top, modifying its pneumatophore as a stopper of wine bottle or as a buoy of fishing net. In Malaysia, sour young fruit have been processed into jam for eating with curry. Its flower has been eaten with chili sauce and squeezed flower juice has been use as ingredient in antidiuretic drug formulations. Furthermore, its flower can be ground with rice and used as poultice for healing the small pox. Leaf of cork tree has been ground and swaddled on head when blood pressure in head is high. Peel of the ripe fruit has used as anthelmintic. Burmese and Indian have applied cork tree as poultice for wounds and bruised wound. Malayan has used peel of mature fruit as anthelmintic and used smashed leaves to heal hemorrhagic urinate symptom and smallpox [1,2].

Phenolic compound such as gallic acid and flavonoids (luteolin and luteolin-7-O-glucoside) are the main substances that found in cork tree which their amount is varied in each part of the plant [3,4]. Generally, type of extracting solvent has effect on total phenolic compound amount in the extract. From our previous study, the highest amount of the substance was found in methanolic extract and followed by that of water extract [5]. However, general approaches in using herbal drug are either brewing in boiling water (in form of tea) or drinking their juice. Herbal tea prepared from cork tree has been reported [6-9]. Additioanally, the hepatoprotective activity of cork tree extract tested in HepG2 cell against ethanol toxic has been presented [10]. From many advantages of cork tree as mentioned above, it is interesting to study possibility of processing cork tree into dietary supplement to enrich its usability in daily life.

In this study, each part of cork tree, e.g. calyx, flower, stamen, pneumatopore, fruit and leaf, was dried and extracted by using boil water. The dry water extracts were prepared with spray drying and freeze drying methods. Morphology and thermal behavior of the water extracts were measured. Thereafter, the total phenolic compound amount and antioxidant activities were determined.

MATERAILS AND METHODS

Materials

Different parts of cork tree were collected from the cork tree in mangrove forest located in Aumpawa, Samutsongkhram province, Thailand. $ABTS^{2-}$ (2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate)) was obtained as sulfonic acid from Sigma (St.Louis, USA). Trolox, (+/-)–6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, 97%, was purchased from Aldrich (Steinheim, Germany). Potassium persulfate, $FeSO_4$ x $7H_2O$, and sodium acetate from Asia Pacific Specialty Chemicals Limited (Seven Hills, Australia). Sodium carbonate was purchased from Ajax Finechem (Seven Hills, Australia). Folin-Ciocalteu reagent and $FeCl_3$ x $6H_2O$ was purchased from CarLo ErbaReagenti (Milano, Italy). 2,4,6-tri-pyridyl-s-triazine (TPTZ) and Gallic acid were purchased from Fluka Chemie GmbH (Switzerland). L-ascorbic acid, and methanol (HPLC grade) from Fisher Scientific UK Limited

(Loughborough, UK), glacial acetic acid, acetone (AR grade), concentrated hydrochloric acid, absolute ethanol and methanol were purchased from Merck (Darmstadt, Germany). Bidistilled water was produced by our laboratory.

METHODS

Water extracts preparations

Different parts of cork tree, e.g. calyx, flower, stamen, pneumatopore, fruit and leaf, were shredded using knife and were then dried in hot air oven at 60°C for 3 days. An accurate amount of each dried parts was then brewed in boiling water, with the ratio of dried plant to water of 1:4, for 30 min. Subsequently, the obtained solution was collected by filtering via filter paper and was separated in to two portions to process into powder by two different drying techniques, e.g. freeze-drying and spray-drying as the following method. The dried extracts were then analyzed the total phenolic compound amount and antioxidant activity. Juice obtained from squeezing fresh parts of cork tree was also dried using the same techniques and analyzed.

Freeze drying method

Solution of the extracts and squeezed juice were frozen at -20°C in refrigerator and dried using freeze dryer (type 77560–01, Labconco, Missouri, USA) for 72 h. Sample temperature was set at -40°C and the pressure was set less than 0.3 mPa.

Spray drying method

Solution of the extracts and squeezed juice were dried using spray dryer (Minispray Dryer, Büchi 190, Switzerland) with 130°C of an inlet air temperature and 80°C of an outlet air temperature.

Morphology of the extracts

Morphology of the water extracts and squeezed juice of the various parts of cork tree that dried by using spray-drying technique or freeze drying technique were observed using scanning electron microscope (SEM: Maxim 200 Cam scan, Cambridge, England). Briefly, the samples were strewed on carbon double adhesive that adhere to aluminum foil which was stuck on metal stub before coating with gold. The test was performed using an accelerating voltage of 1.5 KeV. Seed and leaf extracts which were extracted by using extracting medium containing different ratios of ethanol, 25%, 50% and 75% v/v, were also spray-dried and characterized.

Differential scanning calorimetry (DSC) study

Thermal behavior of the spray-dried sample extracts was equipped using differential scanning calorimeter (Pyris Sapphire DSC, Standard 115V, Perkin Elmer instruments, Japan). Approximately 2 mg of each dried water extracts was weighed into aluminum pan and then was sealed using pan sealer. DSC thermograms were determined in the temperature ranges

of 25-200°C with increasing rate of 10°C/min. The test was performed under the atmosphere of nitrogen gas. An empty aluminum pan was used as the reference.

Determination of total phenolic compound amount

Determination of total phenolic compound was performed using the method describes previously [11]. Briefly, the extracts were dissolved in distilled water until the clear solution was obtained. The solution was measured the amount of total phenolic compound using Follin-Ciocalteu technique. About 1 mL of solution was diluted using 7 mL of distilled water. Thereafter, 1 mL of Follin-Ciocalteu's reagent and 1 mL of sodium bicarbonate (10% w/v) were added into the solution. The mixture was mixed homogenously using vortex and was placed at room temperature for 1 hr before measuring by UV-Vis spectrophotometer at 760 nm. Gallic acid was used as standard substance. Juice obtained from squeezing fresh parts of cork tree was also analyzed.

Antioxidant activity measurement

In this study, two techniques using to measure the antioxidant activity of the cork tree extract, e.g. TEAC method and FRAP (Ferric Reducing Antioxidant Power) method, were performed as the following methods.

TEAC method

Reagent solutions preparation

Antioxidant activity of the extract was investigated using TEAC method (modified from Re, et.al., 1999). An $ABTS^+$ solution was prepared as the method described previously [12]. Firstly, 7 mM $ABTS^+$ solution was mixed with 4.9 mM potassium persulfate by an equal volume. The solution was kept protecting from light and was stored at room temperature for 14-16 hr. Subsequently, formation of $ABTS^+$ was checked by using UV-Vis spectrophotometer (Agilent 8453E UV-Visible spectroscopy System) at 734 nm. The $ABTS^+$ solution was then diluted by adding water to obtained absorbance value approximately 0.7±0.2 at room temperature.

Calibration curve of Trolox

The calibration curve of Trolox, the standard substance, was prepared in the range of 0.00-17.27 x 10^{-3} mg/mL. Thereafter, 50 µL of the standard solution with different concentrations was mixed with 3 mL $ABTS^+$ solution and was then left for 6 min before measuring the absorbance value at 734 nm. The test of each concentration was performed quadruplicated. Distilled water was used as solvent for dissolving sample extract. The absorbance of solvent was determined the same as trolox solution to be used as control. Antioxidant capacity of Trolox that inhibit $ABTS^+$ in each standard solution was calculated in terms of % inhibition as the following equation.

$$\% \text{ inhibition} = ((Abs_{solvent} - Abs_{compound})/Abs_{solvent}) \times 100 \quad \text{.....(1)}$$

Measurement of the antioxidant capacity of the sample extracts, luteolin and luteolin-7-O-glucoside.

The extracts were dissolved in distilled water to obtain the concentration ranges as shown in Table 1. Squeezed juice from fresh plant was also diluted using distilled water. Concentration of luteolin and luteolin-7-O-glucoside were prepared in the ranges of 0-50 µg/50 µL. The sample solution (50 µL) was mixed with $ABTS^+$ solution (3mL) and was measured absorbance value at 734 nm after 6 min of mixing. The test was performed quadruplicated. Calculation of antioxidant capacity of the sample extracts was performed using equation (1) to obtain % inhibition of sample.

Calculation of TEAC (Trolox Equivalent Antioxidant Capacity) was conducted as equation (2).

$$TEAC = \frac{\%\ \text{inhibition of sample}}{\%\text{inhibition of trolox}} \qquad .....(2)$$

% Inhibition of each sample solutions was plotted with its concentrations. Linear equation was calculated using software. Concentration having 50% inhibition (IC_{50}) and TEAC of the same concentration were calculated from equation 2.

FRAP method

Reagent solutions preparation

All reagents were prepared using bidistilled water. FRAP reagent was prepared by mixing 0.3 mol/L sodium acetate buffer pH 3.6 (100 mL), 0.01 mol/L TPTZ in 0.04 mol/L HCl (10 mL) and 0.02 mol/L $FeCl_3.6H_2O$ (10 mL). Standard solutions of $FeSO_4.7H_2O$ were prepared in the concentration ranges of 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mM in 50% methanol. Gallic acid solutions were prepared in methanol in the concentration ranges of 0.02, 0.03, 0.04, 0.05 and 0.06 mg/mL to be used as standard antioxidant. Sample solutions of the crude water extracts and squeezed juice from various parts of cork tree were prepared in the concentration ranges as shown in Table 1.

Calibration curve of Fe^{2+}

Briefly, 150 µL of each standard solution of $FeSO_4.7H_2O$ was mixed with 4.5 mL of the FRAP reagent and 450 µL of bidistilled water. The mixtures were left for 30 min before detecting the absorbance at 595 nm. The test was performed quadruplicated. Blank solution was prepared by using 50% methanol instead of the $FeSO_4.7H_2O$ standard solution. Calibration curve was plotted between the concentration of $FeSO_4.7H_2O$ and the average value of the measured absorbance of each mixture. Linear equation and coefficient of the determination (r^2) were calculated from the calibration curve.

Measurement of the ferric reducing ability of the sample extracts and gallic acid

To measure the ferric reducing ability, 150 µL of each sample solutions or gallic acid solutions was mixed with 4.5 mL of the FRAP reagent and 450 µL of bidistilled water and was then left for 30 min before measuring the absorbance at 595 nm. Blank solutions were prepared by mixing 4.5 mL blank-FRAP reagent (prepared by mixing 110 mL of 0.3 mol/L sodium acetate buffer (pH 3.6) and 10 mL of 0.01 mol/L TPTZ in 0.04 mol/L HCl) with 450 µL of bidistilled water and 150 µL of each sample solutions. Ferric reducing ability of the sample substance was reported as the equivalent concentration of $FeSO_4.7H_2O$ that calculated from the equation obtained from calibration curve of Fe^{2+} and expressing in terms of mM of Fe^{2+} reduced per µg of dried sample.

Calculation of antioxidant capacity of the sample extracts

The antioxidant capacity of the extracts was calculated in terms of GEAC (Gallic acid Equivalent Antioxidant Capacity) by comparing the ferric reducing ability from FRAP assay of each extracts with gallic acid at the same concentration. The calculation was performed as equation (3).

$$GEAC = \frac{\text{Ferric reducing ability of the extract}}{\text{Ferric reducing ability of gallic acid}} \quad \text{..... (3)}$$

GEAC value of each sample solutions was plotted as the same as TEAC. Linear equation was calculated and the IC_{50} and GEAC of this concentration were calculated.

RESULTS AND DISCUSSION

Morphology of the spray-dried powder of the extracts and squeezed juice of various parts of cork tree were demonstrated as Fig. 1 and 2, respectively. The size of the dry particles of all extracts was widely distributed in the ranges of 2-10 µm. Droplet formation from atomizer of spray dryer and rapid water removal with high temperature with the short time resulted in the spherical formation of the particle. The particles were irregularly spherical shape with obviously shrinkage but the least shrinkage was observed for the leaf extract and dried powder of squeezed juice of leaf. This different character of the leaf extract particle comparing to the others might be caused by the high chlorophyll consisted in the leaf that could increase viscosity of the solution and resulted the less shrinkage of the particles after spry drying process. Typically, the incorporation of solid carrier such as colloidal silicon dioxide, maltodextrin or starch has been employed to stabilize the spherical nature of the spray dried products. The higher magnification for spray-dried powder of pneumatophore indicated the smooth surface of the obtained particles. During drying with spray dryer, the expansion of the droplet was occurred owing to the heat exposure and then the particle was cooled down with the shrinkage of the particle too. There was no pore formation or breakage in the particle surface. The particles prepared from calyx and pneumatophore were smaller than the others. The solid content in filtrate prepared from these two parts of cork tree should be lower than that of the other parts therefore the smaller particles were obtained from the dilute solution after spray drying. Morphology of the freeze-dried squeezed juice of stamen, leaf, calyx and fruit was exhibited as irregular

plate-like shape as shown in Fig. 3. The rather large particles were obtained from the calyx extract and those of stamen were smallest. Since the particle formation was obtained from the sublimation of water from the filtrate during freeze drying, the nucleation of particle from the dilute filtrate could form systematically than the concentrate one.

SEM images of ethanol extract of seed and leaf of cork tree were presented in Fig. 4. Amount of ethanol in extracting medium affected the morphology of the spray-dried powder of the extracts. High aggregated pattern of spray dried powder of the seed extracts was observed when the ratio of ethanol was higher. In the contrary, lower aggregation of the leaf extract powder was observed when extracted using higher ratio of ethanol. Aggregation of the spray-dried powder extract might be caused by an increment of viscosity of the spray solution. These results might suggest that compounds containing in seed and leaf of the cork tree was different.

Thermal behavior of the extracts was studied using DSC. Thermogram of almost extracts exhibited two endothermic peaks around 60°C and 160°C except for that of the leaf extract which its endothermic peak exhibited around 130-140°C (Fig. 5). The water soluble phenolic compounds were generally low and their melting point was in high temperature range. Endothermic peak exhibited in DSC thermograms of each cork tree extracts might belong to other water soluble substances. However, the result couldn't be clearly discussed since melting temperature of gallic acid is 250 °C [13] which did not include in the ranges of the operating temperature.

Yield (%w/w) of the water extracts and amount of total phenolic compounds calculated in term of amount of gallic acid contained in 100 g of the extracts and 100 g of each dried parts of cork tree is shown in Table 2. For the fresh plant, 100% yield was considered for squeezed juice which its amount of total phenolic compounds calculated from dried-tree was not done. Considering in terms of drying technique, the extracts obtained from freeze-drying method containing higher yield of the extracts and also higher amount of total phenolic compounds than the extracts that were obtained from spray-drying method. The highest yield of the extract was found in stamen (14.81% w/w from spray-drying and 19% w/w from freeze-drying) and followed by calyx of fruit and flower. The higher yield tended to represent the higher amount of the total phenolic compounds as shown in Table 2. Comparing to the fresh plant, extracting process affected to the reduction of the phenolic compound content. Freeze-dried crude extracts obtained from fresh plant significantly had higher phenolic content than that of the extracts dried by the same technique. Highest amount of the total phenolic compounds was found in stamen (26.62% w/w) and following by that of fresh fruit (23.38% w/w), calyx (19.4% w/w) and leaf (11.89% w/w), respectively. In this study, fresh plant of the other parts was not measured because their juice could not be squeezed.

Effect of the drying method on the amount of total phenolic compound in other plants has been reported previously. Drying method involving with thermal process resulted in highly reduction of the phenolic compound content in ginger whereas the non-thermal process such as freeze-drying method could gain more phenolic compound content, significantly [14]. Comparing between thermal drying techniques, e.g. spray drying (145°C)

and vacuum drying (40°C), the technique operating with higher temperature significantly promoted the loss of the phenolic compound [15].

Antioxidant activities of the extracts in terms of IC_{50}, and TEAC value were shown in Table 3. According to previous study that different antioxidant activity was observed when measuring by the different techniques [16]. Therefore, two or more techniques should be done to confirm the result. In this study, TEAC and FRAP methods were employed. Although the thermal drying technique results the loss of the phenolic compound, the converse effect was observed for antioxidant activities measured from TEAC method. The boil water extracts dried by spray drying technique showed higher antioxidant activities than that of the extracts of the same part dried by freeze drying technique as shown in Table 3. For the spray-dried boil water extracts of the stamen, calyx and leaf exhibited the medium antioxidant activities calculated in term of TEAC value which were in the ranges of 0.66-0.61 comparing to the standard substance, Trolox, which its TEAC value was equal to 1. TEAC of the spray-dried extracts from the other part was in the ranges of 0.42-0.30. Whereas the TEAC value of the freeze-dried extracts of stamen, calyx and leaf were in the ranges of 0.50-0.23 and that of the other parts were in ranges of 0.26-0.10. Other pure compounds, e.g. luteolin and luteolin-7-O-glucoside had TEAC value approximately 0.015 and 0.011, respectively. From thermal drying technique using higher temperature, the reduction of total phenolic compound but increment of an antioxidant activity was consistent to the previous study [15]. TEAC value of the spray-dried squeezed juice obtained from fresh fruit and fresh leaf were 0.49 and 0.35, respectively. Whereas TEAC value of the freeze-dried juice of stamen, fruit and calyx was approximately 0.58 and that of leaf was 0.35.

The different trend was observed from the FRAP method. GEAC value and IC_{50} of the dried extract from boiling and squeeze juice are shown in Table 4. GEAC value of the extracts obtained by freeze-drying method was higher than that of obtained by spray-drying method. GEAC value of the spray-dried boil water extracts of stamen, calyx and leaf was in the ranges of 0.23-0.19 whereas the other part had lower GEAC value with in the ranges of 0.10-0.15. The freeze-dried boil water extracts of stamen, calyx and meat of fruit was in the ranges of 0.23-0.20 whereas that of the other part was in the ranges of 0.06-0.12. Spray-dried juice of fresh fruit and leaf had GEAC value of 0.17 and 0.11, respectively. The GEAC of freeze-dried juice of fresh stamen, calyx and fruit was in the ranges of 0.23-0.20 and that of fresh leaf was 0.16. Antioxidant activities of the extracts and the squeeze juice in terms of GEAC value was rather low comparing to that of pure gallic acid (GEAC value equal to 1). Since water solubility of gallic acid is approximately 11.5 mg/mL [17] and its solubility can be increased by temperature [18,19]. However, its water solubility is very poor, therefore, amount of the gallic acid extracted by boiled water was low and then led to low level of antioxidant activities.

CONCLUSION

Morphology of the spray-dried extracts powder of different parts of cork tree was rather similar except that of the leaf extract. The difference of the leaf extract comparing to the others was also observed in thermal properties study. Drying method had effect on total phenolic compound amount containing in boil water extracts of various parts of cork tree. Thermal process resulted in the reduction of the total phenolic content of both of the

ISSN: 0975-8585

extracts from dried plant and squeezed juice from fresh plant. Fresh plant contained higher amount of the phenolic compound than that of dried plant. The highest total phenolic content was found in stamen followed by calyx and leaf. Antioxidant activities measuring by TEAC and FRAP methods of the boil water extracts and squeezed juice that were dried by different techniques was rather different. Therefore, further experiment should be done to confirm these results.

Figure 1 SEM images of spray-dried powder extracts (A: Stamen, B: Seed, C: Leaf, D: Calyx, E: Fruit and F: Pneumatophore) and spray-dried powder of squeezed juice (G: fruit, H: leaf) of cork tree at magnification of 500.

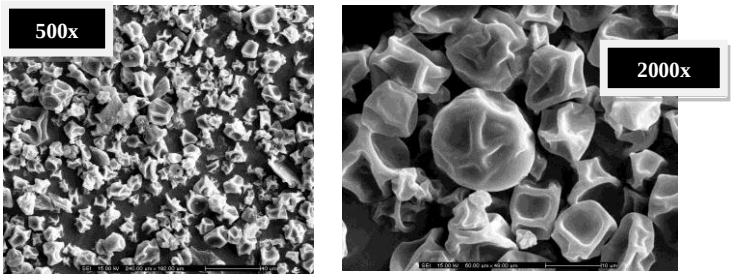


Figure 2 SEM images of spray-dried powder of pneumatophore extract of cork tree at different magnifications.

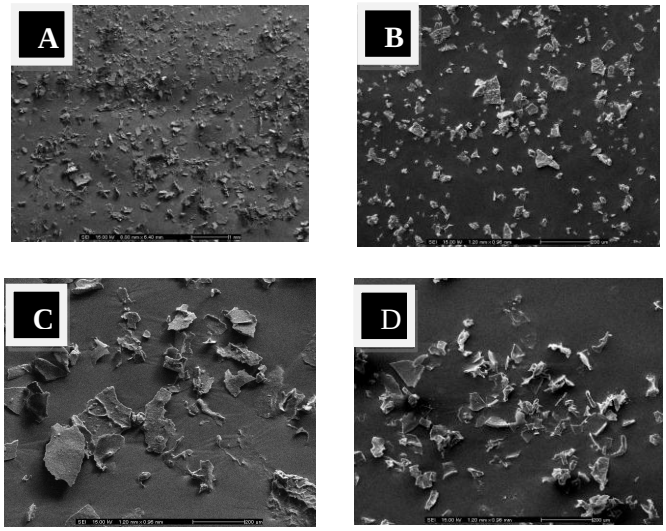
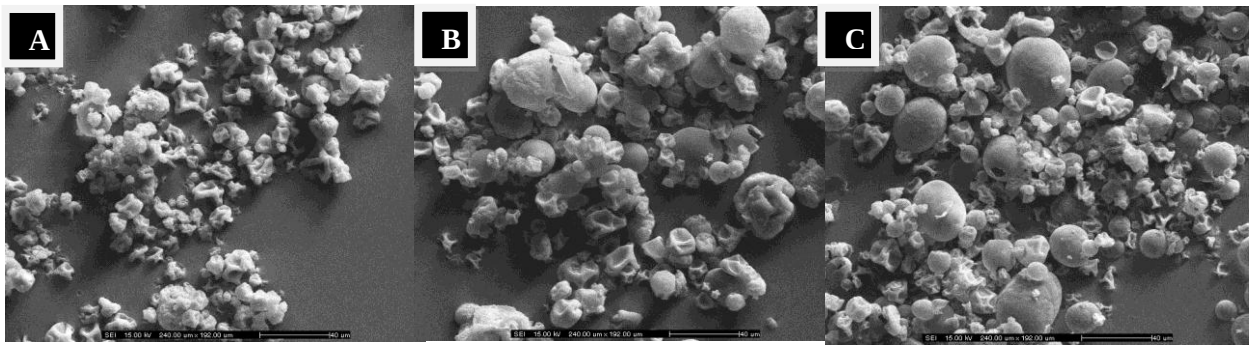
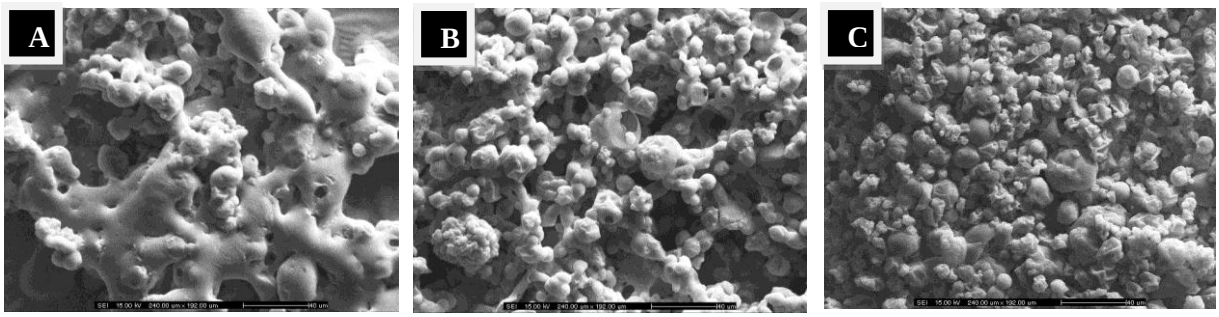


Figure 3 SEM images of freeze-dried squeezed juice (A: Stamen, B: Leaf, C: Calyx and D: Fruit) of cork tree at magnification of 100.



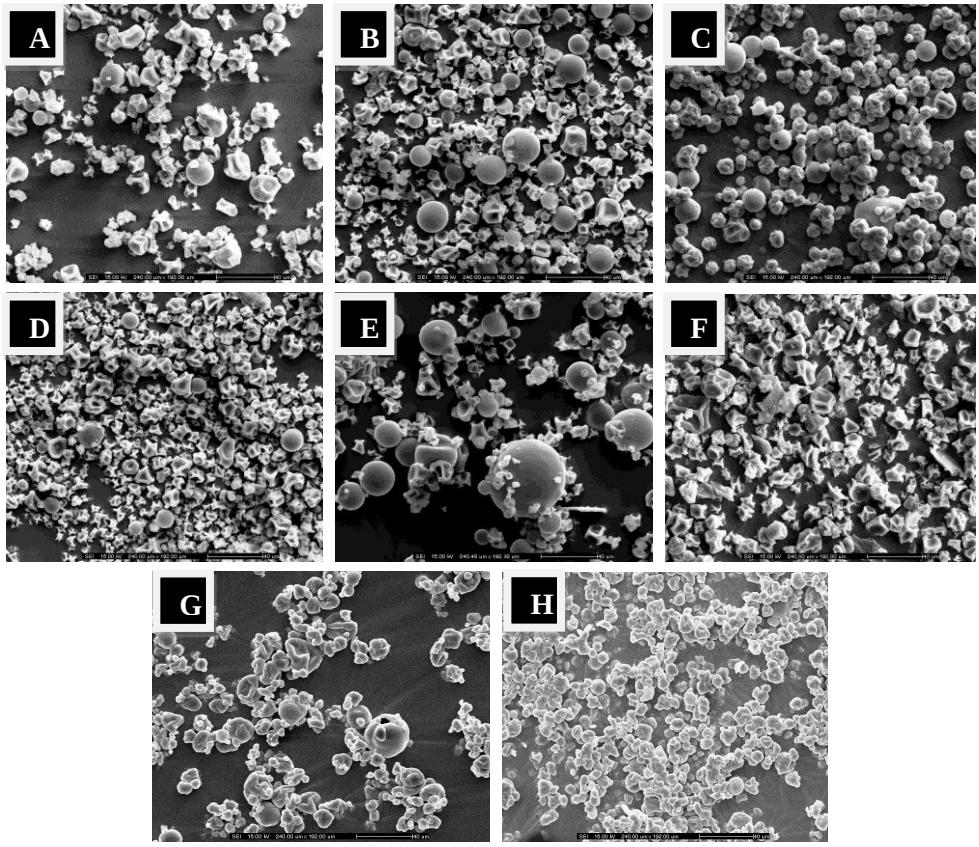
Seed extract

ISSN: 0975-8585



Leaf extract

Figure 4 SEM images of spray-dried powder extracts of seed (upper row) and leaf (lower row) of cork tree extracted with different amount of ethanol (A: 25%, B: 50% and C: 75% v/v) at magnification of 500.



ISSN: 0975-8585

Figure 5 DSC curve of spray-dried powder extracts (A: Stamen and B: Leaf) of cork tree.

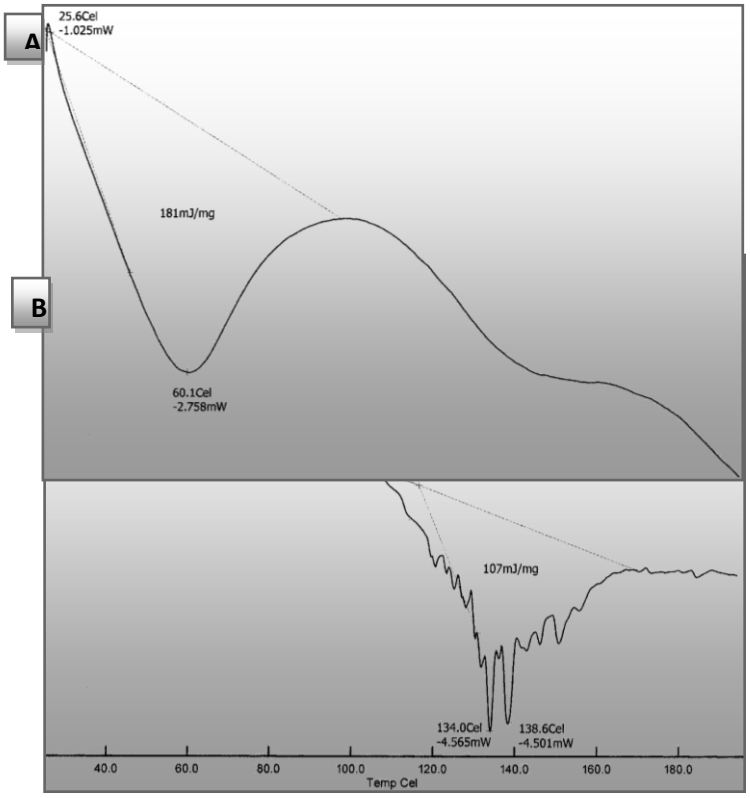


Table 1 Concentration ranges of the solutions of the extracts and squeezed juice using in ABTS and FRAP method.

Type of cork tree extract	Concentration range	
	ABTS (µg/ 50µL)	FRAP(µg/ 150µL)
Boil water extracts		
Spray-dried		
Stamen	0-25	0-30
Calyxs of flower	0-27.5	0-40
Fruit	0-55	0-70
Persistent calyxs of fruit	0-50	0-80
Seeds	0-50	0-45
pneumatophores	0-45	0-45
Leaf	0-25	0-30
Freeze-dried		
Stamen	0-40	0-30
Calyxs of flower	0-32.5	0-30
Fruit	0-82.5	0-30
Persistent calyxs of fruit	0-75	0-60
Seeds	0-200	0-108
Pneumatophores	0-65	0-60
Squeezed juice		
Spray-dried		
Fresh fruit	0-50	0-45
Fresh leaf	0-35	0-60
Freeze-dried		
Fresh stamen	0-30	0-30

ISSN: 0975-8585

Fresh flower calyx	0-30	0-35
Fresh fruit	0-50	0-30
Fresh leaf	0-27.5	0-60

Table 2 Yield (%) of the boiled water extracts from different parts of cork tree and total phenolic compound amount calculated in terms of gallic acid (g) per 100 g of dried-plant and crude extract

Type of extracts	**% yield**	**Total phenolic compound** (g/100g dried-tree as Gallic acid)	**Total phenolic compound** (g/100g crude extract as Gallic acid)
Boil water extracts			
Spray-dried			
Stamen	14.81	2.60±0.06	17.54±0.38
Calyxs of flower	2.31	0.19±0.00	8.15±0.21
Fruit	2.89	0.40±0.01	13.73±0.35
Persistent calyxs of fruit	5.26	0.64±0.03	12.23±0.63
Seeds	4.33	0.05±0.01	1.16±0.26
Pneumatophores	2.32	0.11±0.02	4.50±0.82
Leaf	5.51	0.86±0.00	15.66±0.09
Freeze-dried			
Stamen	19.00	2.63+0.02	13.82+0.11
Calyxs of flower	9.94	1.56+0.02	15.71+0.18
Fruit	9.81	0.76+0.03	7.79+0.30
Persistent calyxs of fruit	6.86	0.74+0.01	10.81+0.16
Seeds	3.02	0.17+0.00	5.75+0.04
Pneumatophores	5.06	0.68+0.03	13.50+0.58
Squeezed juice			
Freeze-dried			
Fresh stamen	-	-	26.62+1.31
Fresh flower calyx	-	-	19.40+0.21
Fresh fruit	-	-	23.38+1.68
Fresh leaf	-	-	11.89+1.26

Note: Since some part of the fresh plant could not be squeezed, their value did not show in the Table.

Table 3 Equation parameters (slope, intercept and r^2) and antioxidant activities in terms of IC_{50} and its TEAC value measured by using ABTS method

Type of substance and extracts	**Equations**			**IC_{50}** (μg/ 50μL)	**TEAC**
	slope [a]	intercept	r^2		
Trolox	5.0248	-0.9708	0.9985	10.14	1
Luteolin	0.0397	3.3283	0.9960	1175.61	0.015
Luteolin-7-glycoside	0.0233	3.0112	0.9923	2016.69	0.011
Boil water extract					
Spray-dried					
Stamen	3.1872	2.0029	0.9971	15.06	0.64
Calyxs of flower	3.0022	4.2414	0.9881	15.24	0.61
Fruit	1.5222	4.6869	0.9874	29.77	0.31
Persistent calyxs of fruit	1.4909	2.7496	0.9949	31.69	0.30
Seeds	1.7320	4.1605	0.9914	26.47	0.35
Pneumatophores	2.0434	6.5042	0.9825	21.29	0.42
Leaf	3.2527	3.7305	0.9921	14.22	0.66

ISSN: 0975-8585

Freeze-dried					
Stamen	2.0755	4.417	0.9869	21.96	0.42
Calyxs of flower	2.437	4.915	0.9829	18.50	0.50
Fruit	1.0503	1.5361	0.9974	46.14	0.21
Persistent calyxs of fruit	1.0968	6.2168	0.9737	39.92	0.23
Seeds	0.4256	9.3644	0.9565	95.48	0.10
Pneumatophores	1.2652	5.5584	0.9778	35.13	0.26
Squeezed juice					
Spray-dried					
Fresh fruit	2.422	3.5181	0.9935	19.194	0.49
Fresh leaf	1.7129	6.0037	0.9787	25.69	0.35
Freeze-dried					
Fresh stamen	2.8490	4.6224	0.9882	15.928	0.58
Fresh flower calyxs	2.8058	3.9934	0.9892	16.40	0.57
Fresh fruit	2.8669	3.9734	0.9908	16.05	0.58
Fresh leaf	1.7237	3.1967	0.9920	27.15	0.35

Table 4 Equation parameters (slope, intercept and r^2) and antioxidant activities in terms of IC_{50} and its GEAC value measured by using FRAP method

Type of the extracts	Equation parameters			IC_{50} (mM/50 µg of dried sample)	GEAC
	slope [a]	intercept	r^2		
$FeSO_4$	3.7133	0.0269	0.9958	-	-
Gallic acid	0.1329	0.0172	0.9883	1.7869	1
Boil with water					
Spray dry					
Stamen	0.0264	0.0776	0.9977	0.3691	0.21
Calyxs of flower	0.0248	0.0523	0.9984	0.3408	0.19
Fruit	0.0136	0.0468	0.9996	0.1885	0.11
Persistent calyxs of fruit	0.0115	0.1027	0.9992	0.1753	0.10
Seeds	0.02	0.0436	0.9903	0.2738	0.15
Pneumatophores	0.0184	0.0584	0.9991	0.2562	0.14
Leaf	0.0298	0.0428	0.9972	0.4055	0.23
Freeze dry					
Stamen	0.0280	0.0986	0.9968	0.3963	0.22
Calyxs of flower	0.0266	0.0526	0.9977	0.3651	0.20
Fruit	0.0314	-0.0178	0.9978	0.4108	0.23
Persistent calyxs of fruit	0.0151	0.0437	0.9944	0.2078	0.12
Seeds	0.0080	0.0116	0.9993	0.1036	0.06
Pneumatophores	0.0137	0.0699	0.9999	0.1961	0.11
Leaf	0.0146	0.0604	0.9952	0.2056	0.12
Squeeze juice					
Spray-dried					
Fresh fruit	0.0230	0.0229	0.9986	0.3086	0.17
Fresh leaf	0.0133	0.0644	0.9962	0.1892	0.11
Freeze-dried					
Fresh stamen	0.0280	0.0986	0.9968	0.3963	0.22
Fresh flower calyxs	0.0266	0.0526	0.9977	0.3651	0.20

Fresh fruit	0.0314	-0.0178	0.9978	0.4108	0.23
Fresh leaf	0.0146	0.0604	0.9952	0.2056	0.16

ACKNOWLEDGEMENTS

This study was kindly supported by Research and Development Institute of Silpakorn University and Faculty of Pharmacy, Silpakorn University.

REFERENCES

[1] Duke JA. 1983. Sonneratia caseolaris (L.) Engl. [online]. (Accessed 23/05/2011). Avialable from: http:// www.hort.purdue.edu/newcrop /duke_energy/ Sonneratia_ caseolaris. html.
[2] Lim KKP, Murphy DH, Morgany T, Sivasothi N, Ng PKL, Soong BC, Tan HTW, Tan KS, Tan TK. 2001. Guide to the mangrove of Singapore. [online]. (Accessed 23/05/2011). Available from: http://mangrove.nus.edu.sg/guidebooks/text/1074.htm
[3] Sadhu SK, Ahmed F, Ohtsuki T, Ishibashi M. J Nat Med 2006;60:264–265.
[4] Wu SB, Wen Y, Li XW, Zhao Y, Zhao Z, Hu JF. Biochem Syst Eco 2009;37:1–5.
[5] Limmatvapirat C, Sukjindasathein S, Charoenteeraboon J, Wetwitayaklung P, Phaechamud T. Proceedings of the 2nd Silpakorn University Research Fair, 23 November, 2009, Nakorn Pathom, Thailand.
[6] Phaechamud T, Limmatvapirat C. The Third International Conference on Natural Products for Health and Beauty. 16-18 March 2011. Bangkok, Thailand, p.159.
[7] Phaechamud T, Chuekaew A, Yodkhum K Proceedings of The 3rd Annual Northeast Pharmacy Research Conference, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Thailand, February 21-26, 2011, pp. 133-139.
[8] Phaechamud T, Limmatvapirat C. Proceedings of The 3rd Annual Northeast Pharmacy Research Conference, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Thailand, February 21-26, 2011, pp. 42-45.
[9] Phuagphong P, Punpae S, Phaechamud T. The 7th Conference of Asia Society for Mitochondrial Research and Medicine [ASMRM] and The 10th Conference of Japanese Society of Mitichondrial Research and Medicine [J-mit]. December 16-18, 2010, Fukuoka International Congress Center, Fukuoka city, Japan, p. 118.
[10] Prapongsena N, Phaechamud T, Charoenteeraboon J. The 3rd Annual Northeast Pharmacy Research Conference. Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Thailand, February 21-26, 2011, pp. 21-26.
[11] Kumazawa S, Taniguchi M, Suzuki Y, Shimura M, Kwon MS, Nakayama T. J Agri Food Chem 2002;50:373-377.
[12] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Free Radl Bio Med 1999;26:1231-1237.
[13] Wikimedia Foundation, Inc. 2011. Gallic acid. [online]. (Accessed 06/06/2011). Avialable from: http://en.wikipedia.org/wiki/Gallic_acid
[14] Chan EW, Lim YY, Wong SK, Lim KK, Tan SP, Lianto FS, Yong MY. Food Chem 2009;113:166–172.
[15] Ramamoorthy PK, Bono A. J Eng Sci Tech 2007;2(1):70 – 80.
[16] Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. J Food Comp Anal 2006;19:669–675.

[17] O'Neil MJ, Smith A, Heckelman PE, Obenchain JR, Gallipeau JAR, D'Arecca MA. The Merck index 13th edn, Merck research laboratories, NJ, 2001, p. 261.
[18] Daneshfar A, Ghaziaskar HS, Homayoun N. J Chem Eng Dat 2008;53:776–778.
[19] Mota FL, Queimada AJ, Pinho SP, Macedo EA. Ind Eng Chem Res 2008;47:5182–5189.