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Section G - Agricultural Science

G_G0019: A COMPARATIVE STUDY ON ANTIOXIDANT ACTIVITIES IN THE DIFFERENT SOLVENT EXTRACTS OF *Garcinia mangostana* L. SEED USING CHEMICAL ASSAYS

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Abstract: From the clean production of commercial freeze-dried mangosteen juice powder of Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi (Bangkhuntien), the mangosteen (*Garcinia mangostana* L.) seed was selected as the one part of our ongoing research to examine the active compounds such as phenolics and flavonoids for adding value and using as natural antioxidants. In this study, a comparative antioxidant activities of the different solvent extracts with different polarities (such as water, methanol, ethanol, acetonitrile, acetone, dichloromethane, ethyl acetate and *n*-hexane) obtained from *Garcinia mangostana* L. seed was determined. The chemical assays were performed to evaluate the antioxidant activities based on Folin-Ciocalteu reagent assay (total phenolics), aluminium chloride colorimetric assay (total flavonoids), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and 2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate (ABTS) radical cation decolorization assays. Among the different extracts studied, the mangosteen seed extract with dichloromethane was observed to have highest antioxidant activity. The extract gave highest total phenolics and total flavonoids value of 13.27 ± 0.01 mg gallic acid equivalent (GAE)/100 g crude extract (CE) and 45.26 ± 0.04 mg rutin equivalent (RE)/100 g CE, while IC_{50} showed strong scavenging activity values of 57.07 ± 0.06 μ g/ml and 46.24 ± 0.05 μ g/ml for ABTS and DPPH assays, respectively. Furthermore, the results showed that there is a strong correlation ($R, 0.99^{**}$) between total phenolic with total flavonoids contents of the extracting solvents at the 95% confidential level. From these results it can be inferred that dichloromethane was the most suitable solvent to extract the antioxidant compounds from mangosteen seed.

Introduction: Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer.¹ Mangosteen (*Garcinia mangostana* L.), locally called as Mung-Koud, is a one of the important exported fruit of Thailand and Thai agricultural economy. The mangosteen-fruit is dark purple or reddish, with white, soft and juicy edible pulp with a slightly acid, sweet flavor and a pleasant aroma.² Phytochemical studies on various parts of *Garcinia mangostana* L. revealed that it contains the major bioactive compounds such as phenolic acid, prenylated xanthone derivatives, anthocyanins, and procyanidins.³⁻⁵ Widely reported the *Garcinia mangostana* L. were extracted by various solvents include the acetone⁶, ethanol⁷, ethyl acetate⁸, hexane⁹, methanol.¹⁰ However, research on their antioxidant compounds from the mangosteen seed extract with different polarity has been scarce. Difference extraction solvents may have a difference active constituents and antioxidant characters. Therefore, the objectives of this study aim to find the most effective solvent to extract antioxidant compounds (such as phenolics and flavonoids) from *Garcinia mangostana* L. seed. The ABTS and DPPH method was used to determine the free radical scavenging of each extracting solvents. The correlation of total phenolic with total flavonoid contents in the mangosteen seed extracts were also was investigated.

From the clean production of commercial freeze-dried mangosteen powder of Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi (Bangkhuntien), the edible part of mangosteen fruit was produced a freeze dried mangosteen juice powder and the two waste parts of mangosteen (peel and seed); the peel was extracted and separated the xanthone compounds which are well known for their interesting phytochemical properties and the seed was selected as the last part of our ongoing research to examine the biologically active compounds such as phenolics and flavonoids for adding value and using as natural antioxidants, which make them attractive to the pharmaceutical and medicinal industry.

Methodology: Fresh mangosteen (*Garcinia mangostana* L.) fruits at mature stage were purchased from local markets in Bang KhunThian District, Bangkok Province of Thailand. Upon arrival at the laboratory, samples were thoroughly washed with tap water and manually peeled. The fresh seeds of *Garcinia mangostana* L. were homogenized using a glass grinder. For the extraction, a weighted amount of homogenized samples (0.5g) was extracted continuously with 20 ml of eight different polarities of solvents such as water, methanol, ethanol, acetonitrile, acetone, dichloromethane, ethyl acetate and *n*-hexane. The mixture was sonicated by a Bransonic cleaner and centrifuged at 2000 g for 5 min at room temperature. The supernatant was filtered using Whatman No.1 filter paper. After filtration, the solvent was removed by rotary vacuum evaporator (BÜCHI rotaryvapor R-200, Switzerland). Extracts were dried at 50-60 °C until the constant mass was achieved. Dried crude extracts were stored in amber bottles at 4 °C for prevention from oxidative damage until needed for analysis.

This experiment was carried out to the study the influence of different solvents on the extractability of some of the antioxidant compounds from mangosteen (*Garcinia mangostana* L.) seeds. A Completely Randomized Design (CRD), consisted eight treatments (water, methanol, ethanol, acetonitrile, acetone, dichloromethane, ethyl acetate and *n*-Hexane) and three replicates each was used.

Determination of total phenolics

The Folin-Ciocalteu method of Waterhouse¹¹ was used to estimate total phenolics. The seed extract (500 µl) was diluted with deionized water to 4.5 ml, and 300 µl of Folin-Ciocalteu reagent was added and shaken. After 8 minutes, 900 µl of 20% sodium carbonate solution was added with mixing. The solution was left at 40°C for 30 minutes before reading the absorbance at 765 nm using a UV/Visible spectrophotometer (UVIKON 900/XL/X5, Schott instruments, France). Gallic acid was used as standard, and the results were reported as milligram gallic acid equivalent per 100 gram of crude extracts (mg GAE/100 g CE).

Determination of total flavonoids

Colorimetric aluminum chloride with modified method¹² was used by diluted solution (1 ml) containing flavonoids, 5% (w/w) NaNO₂ (0.7 ml) and 30% (v/v) ethanol (10 ml) were mixed for 5 minutes, and then 10% (w/w) AlCl₃ (0.7 ml) was added and mixed altogether. Six minutes later, 1 M NaOH (5 ml) was added. The solution was then diluted to 25 ml with 30% (v/v) ethanol. After standing for 10 minutes, the absorbance of the solution was measured at 430 nm with a spectrophotometer. A standard curve was plotted using rutin as a standard. Different concentrations of rutin were prepared in 80% ethanol and their absorbance was read at 430 nm using a spectrophotometer. The results were expressed in milligram rutin equivalent per 100 gram of crude extracts (mg RE/100 g CE) by comparison with the rutin standard curve, which was made under the same condition.

DPPH radical scavenging activity

The method of Brand-Williams *et al.*¹³ was used with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and then stored at 4°C in the dark until needed. The working solution was obtained by mixing 10 ml stock

solution with 45 ml methanol to obtain an absorbance of 1.1 ± 0.02 units at 515 nm. The each seed extracts (150 μ l) were allowed to react with 2850 μ l of the DPPH solution for 24 h in the dark at room temperature. Then the absorbance was taken at 515 nm. Additional dilution was needed if the DPPH value measured was over the linear range of the standard curve.

ABTS cation radical scavenging assay

The ABTS method of Rice-Evans *et al.*¹⁴ was modified. The stock ABTS radical cation (ABTS^{•+}) solutions included 7.0 mM ABTS^{•+} solution and 2.45 mM potassium persulphate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 hours in the dark at room temperature. The solution was then diluted by mixing 1 ml ABTS^{•+} solution with 60 ml methanol to obtain an absorbance 1.1 ± 0.02 units at 734 nm. The each seed extracts (150 μ l) were allowed to react with 2850 μ l of diluted ABTS^{•+} solution for 2 h in the dark at room temperature. The decrease of absorbance was recorded. Additional dilution was needed if the ABTS value measured was over the linear range of the standard curve.

Absorbance decreases were calculated as DPPH and ABTS values by IC₅₀ values, indicating the final concentrations of samples required for 50 % reduction of the absorbance of ABTS and DPPH assay, were also calculated.

Statistical methods

All data were presented as mean \pm standard error of the means of three independent experiments, carried out using analysis of variance (ANOVA) and Duncan's multiple comparison tests. Pearson's correlation was used to determine the correlation of data between total flavonoids and total phenolics. Statistical analysis was performed with Excel version 2010 (Microsoft Inc.) and SPSS version 17.0 software.

Results and Discussion: From Table 1 showed total phenolics of mangosteen seed extract ranged from 0.78 ± 0.01 mg GAE/100 g CE for *n*-Hexane to 13.27 ± 0.01 mg GAE/100 g CE for dichloromethane and total flavonoids ranged from 2.66 ± 0.02 mg RE/100 g CE for *n*-Hexane to 45.26 ± 0.04 mg RE/100 g CE for dichloromethane. Mangosteen seed extracted with dichloromethane showed the highest phenolic and flavonoid content. Based on the results obtained, it is possible that several compounds of different polarities may contribute to the antioxidative properties of mangosteen seed extracts. The obtained results of the current investigation are in agreement with Sanaa MM S.¹⁵ and Andrew *et al.*¹⁶ reported that dichloromethane extracts may include flavonoids and phenolic compounds with acids, alcohols, stilbenes, tocopherols and triterpenoids. Due to total flavonoids of all extracts have higher values than total phenolics, we can assume that the flavonoids are the most active compounds of mangosteen seed.

The antioxidant activity as determined by ABTS and DPPH assays are based on the reduction of ABTS and DPPH free radicals of samples. The results of the antioxidant assays have been compared as shown in Table 1. Two methods for measuring antioxidant assays; ABTS and DPPH showed a correlation remarkable behavior. The mean values of the antioxidant assay have been listed, including the inter-individual standard deviations. The results showed all extracts gave the antioxidant activities by ABTS and DPPH assay. DPPH assay better reflects the antioxidant contents in all seed extracted than ABTS assays. All seed extracts contained a considerable amount of antioxidant effect ranged from 57.07 ± 0.06 to 458.44 ± 1.84 and 46.24 ± 0.05 to 371.47 ± 1.49 of the IC₅₀ values (μ g/ml) for ABTS and DPPH assays, respectively. A lower IC₅₀ value indicates a higher antioxidant activity of a compound. It was found that the mangosteen seed extract in dichloromethane possesses the strongest DPPH (IC₅₀, 46.24 ± 0.05 μ g/ml), as well as ABTS radical scavenging (IC₅₀, 57.07 ± 0.06 μ g/ml). In addition, the antioxidant activity was found to be different for different kinds of solvent extracts. However, a similar trend was observed in the study of ABTS and

DPPH radical scavenging activity. The results of this study indicated that mangosteen seed was extracted with less polarity of solvent gave higher free radical scavenging activity than mangosteen seed was extracted with high polarity of solvent. Among eight extraction solvents, dichloromethane could be more effective extraction solvent in recovering highest amounts of antioxidant compounds from mangosteen seed while *n*-Hexane give less effective ($P < 0.05$).

Furthermore, a correlation analysis was performed on total flavonoid and phenolic contents of mangosteen seed extracts. Typical correlation graphs are shown in Figure 1. The correlation between total flavonoids and phenolics in the different extracts of solvents was found to be 0.99** which is highly significant ($P < 0.05$). This indicates that the contents of phenolics exhibited good correlation with flavonoids. Antioxidant activity is found to be linearly proportional with phenolic and flavonoid contents. The obtained results are in accordance with Oktay *et al.*¹⁷ reported a strong positive relationship between total phenolic contents and antioxidant activity, which appears to be the trend in many plant species. The results provided that phenolic acids and flavonoids are the major contributors to the antioxidant property of mangosteen seed extracts. The perceptible correlation among different investigations exhibited that the antioxidant capacity selected in the present investigation are feasible and complementary to the antioxidant activities in a natural antioxidant.

Table 1. Total phenolics, total flavonoids and total antioxidant activity of mangosteen (*Garcinia mangostana* L.) seeds extracted according to different solvents.

Extracts	Total Phenolics*	Total Flavonoids**	Total Antioxidant activity	
			ABTS***	DPPH***
Water	1.21 ± 0.01 ^g	4.11 ± 0.03 ^g	281.31 ± 0.74 ^b	228.31 ± 0.60 ^b
Methanol	2.35 ± 0.01 ^f	8.01 ± 0.04 ^f	136.07 ± 0.19 ^e	110.26 ± 0.15 ^e
Ethanol	2.39 ± 0.02 ^e	8.16 ± 0.06 ^e	137.44 ± 0.20 ^e	111.37 ± 0.16 ^e
Acetonitrile	4.88 ± 0.01 ^c	16.65 ± 0.01 ^c	149.59 ± 1.20 ^d	121.21 ± 0.97 ^d
Acetone	4.12 ± 0.01 ^d	14.06 ± 0.01 ^d	174.32 ± 2.22 ^c	141.25 ± 1.80 ^c
Dichloromethane	13.27 ± 0.01 ^a	45.26 ± 0.04 ^a	57.07 ± 0.06 ^g	46.24 ± 0.05 ^g
Ethyl acetate	9.54 ± 0.01 ^b	32.55 ± 0.03 ^b	64.72 ± 0.14 ^f	52.44 ± 0.11 ^f
<i>n</i> -Hexane	0.78 ± 0.01 ^h	2.66 ± 0.02 ^h	458.44 ± 1.84 ^a	371.47 ± 1.49 ^a

* mg gallic acid equivalent (GAE) / 100 g crude extracts (CE).

** mg rutin equivalent (RE) / 100 g crude extracts (CE).

*** µg/ml, The IC₅₀ values of gallic acid for ABTS and DPPH were 1.06 ± 0.01 and 1.31 ± 0.01 µg/ml, respectively.

Each value in the table is represented as mean ± standard error of the means of three independent experiments (n= 3) with different superscripts in each column, indicate significant difference ($P < 0.05$) analyzed by Duncan's multiple range test.

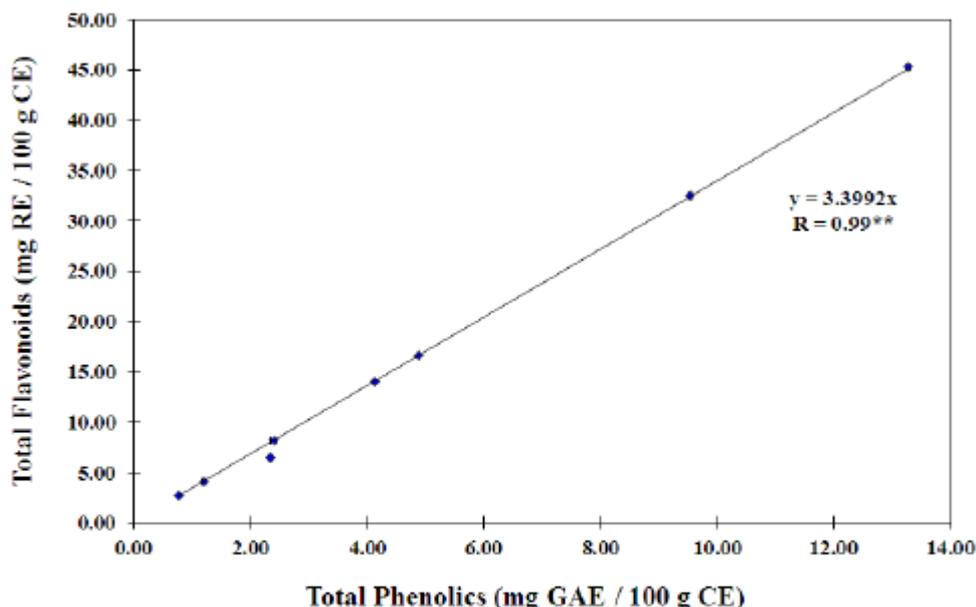


Figure 1. Correlation between the results of total phenolics and total flavonoids for the mangosteen (*Garcinia mangostana* L.) seed extracts (n=8).

Conclusion: Mangosteen seed extracts were carried using eight solvents with different polarity. The extraction of mangosteen seed with dichloromethane showed maximum total phenolics, flavonoids and antioxidant activity. The results indicated the most active compounds in mangosteen seed are flavonoids and all active compounds like to dissolve in less polarity of extraction solvent such as dichloromethane and ethyl acetate. This work was undertaken to explore the potential of waste materials as sources of powerful natural antioxidants.

References:

1. Renaud SC, Gueguen R, Schenker J, d'Houtaud A. *Epidemiology* 1998; 9:184-188.
2. Jung HA, Su BN, Keller WJ, Mehta RG, Kinghorn D. *J Agric Food Chem*. 2006;54:2077-2082.
3. Fu C, Loo AEK, Chia FPP, Huang D. *J Agric and Food Chem*.2007;55:7689-7694.
4. Zadernowski R, Czaplicki S, Naczki M. *Food Chem*. 2009;112:685-689.
5. Chaivisuthangkura A, Malaika Y, Chaovanalikit A, Jaratrungtawee A, Panseeta R, Ratananukul P, Suksamram S. *Fruit Hull. Chromatographia*. 2009;69:315-318.
6. Chen LG, Yang LL, Wang CC. *Food Chem Toxicol*. 2008;46(2):688-693.
7. Destandau E, Toribio A, Lafosse M, Pecher V, Lamy C, André P. *J Chromatogr. A*, 2009;1216(9):1390-1394.
8. Chaivisuthangkura A, Malaikaew Y, Chaovanalikit A, Jaratrungtawee A, Panseeta P, Ratananukul P. *Chromatographia*.2008;69(3-4):315-318.
9. Sakagami Y, Iinuma M, Piyasena KGNP, Dharmaratne HRW. *Phytomedicine*. 2005;12(3):203-208.
10. Chin YW, Jung HA, Chai H, Keller WJ, Kinghorn AD. *Phytochem*. 2008;69(3):754-758.
11. Waterhouse A, Wiseman S, Korver O. *Crit Rev Food Sci Nutr*. 2001;41:387-412.
12. Bushra S, Farooq A, Muhammad A. *Molecules*. 2009;14:2167-2180.

13. Brand-Williams W, Cuvelier ME, Berset C. *Wissenschaft und Technologie* 1995;28: 25-30.
14. Rice-Evans CA, Miller NJ. *Biochem Soc Trans.* 1996;24:790-795.
15. Sanaa MMS. *Int J Agri Biol.* 2007;9(2):220-225.
16. Andrew PJ, David EM, Christine E. *Phytochem Anal.* 1999;10:39-43.
17. Oktay M, Gulcin I, Kufrevioglu OI. *Lebensm-Wiss U-Techol.* 2003;36:263-271.

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