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Phytochemical screening and antioxidant properties of ethanolic crude extract of Som-kwai (*Garcinia pedunculata*)

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Abstract:

Som-kwai (*Garcinia pedunculata*) is widely distributed in the southern part of Thailand, especially in Phuket province, and commonly used as an ingredient in food, and medicinal plant. It shows cardiac stimulant, digestive and laxative properties. The objective of the present study was to explore the phytochemical constituents, and to evaluate the total phenolic and total flavonoid contents and antioxidant activity of the crude extracts from the fruit of *G. pedunculata*. The extraction process was done by maceration method using ethanol as the extracting solvent. The percentage yield of the *G. pedunculata* fruit extract was 24.25 ± 2.70. The phytochemical screening of the crude extract using Shinoda, concentrated sulfuric acid, and Salkowski tests revealed the presence of flavonoids, glycosides, terpenoids, and sterols. Total phenolic and total flavonoid contents were 74.37 ± 6.31 mg gallic acid equivalents/g and 175.90 ± 0.40 mg quercetin equivalents/g using Folin–Ciocalteu and aluminum chloride methods, respectively. In addition, it displayed antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay with an IC_{50} value of 22.40 ± 0.46 mg/ml.

1. Introduction

The rigorous oxidation process occurring in the human body results in the formation of reactive oxygen and nitrogen species (RONS). These reactive species can damage the DNA and lead to the oxidation of lipid and protein in cells causing oxidative stress in the human organism.[1]

Antioxidants remove these free radical intermediates by oxidized themselves, and hence have a physiological function in the body to eventually stop these oxidation reactions. This protects the body from harmful chain reactions. Normally, antioxidant system occurring in human body can scavenge these radicals. However, most human cells do not generate adequate amounts of antioxidants to protect them against oxidative reactions of the produced free radicals.[2] Increasing the intake of synthetic or natural exogenous antioxidants, or both, is therefore essential.

Much attention has been paid in recent years to the use of natural antioxidants from plants. This is because there are studies indicating unwanted or adverse effects that may be associated with the consumption of synthetic antioxidants. It is well known that plants in nature are rich sources of bioactive secondary metabolites, such as flavonoids, glycosides, saponins, terpenes, sterols, tannins, alkaloids and other metabolites.[3] It has been reported that the most of these groups have antioxidant activity. Therefore, currently, attention is focused to the phytochemical screening and antioxidant activity of the various plant extracts.

G. pedunculata belongs to the family Clusiaceae and is endemic to the south eastern regions of Asia such as parts of Myanmar and North-Eastern parts of India. In Thailand, it is widely distributed in the southern part, especially in Phuket province.[4] It is a perennial shrub with a height of about 5-14 meters. The tree has a fluted trunk with short spreading branches. Leaves are lanceolate with prominent mid ribs. The fruit is round with a diameter ranging between 8 cm and 12 cm. It has a juicy interior with edible arils. The mature *G. pedunculata* fruit is greenish yellow and is consumed as a vegetable. Dried pulp of these fruits is used as an astringent, antiscorbutic, cooling, cardiotonic, emollient, antidiarrhoeic, indyspepsia, anti-dysenteric, and in flatulence.[5]

The aims of this study were to perform the phytochemical screening, to determine the total phenolic and flavonoid contents as well as antioxidant activities of *G. pedunculata* extract.

2. Materials and Methods

2.1 Plant materials

G. pedunculata was collected from Phuket, Thailand. Fresh fruits were sliced and dried under sunlight for 7 days. The dried pulps were further heated in a hot air oven at 60 °C for 6 hours and ground into power. The powder was kept at room temperature for further use.

2.2 Reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin-Ciocalteu reagent, Dragendorff reagent, gallic acid, quercetin, iron (III) chloride, aluminium trichloride, sodium carbonate, hydrochloric acid, sulphuric acid, chloroform, potassium hydroxide and magnesium ribbon were purchased from Sigma– Aldrich (St. Louis, USA). Ethanol was supplied from Merck (Darmstadt, Germany). All reagents were prepared using ultrapure water (Milli-Q, USA) in all experiments.

2.3 Preparation of crude extracts

Extraction of *G. pedunculata* fruit was conducted by maceration using an electric stirrer. Fifty grams of dried pulp of *G. pedunculata* fruits were extracted in 100 ml of 90% ethanol for 7 days. The extract was filtered and evaporated using rotary evaporator (40 ^{o}C) under reduced pressure to obtain the crude extracts. This crude extract was used for the phytochemical analysis, total phenolic and flavonoid contents and antioxidant activity.

2.4 Phytochemical screening

The qualitative assay consisted of tests for flavonoids, tannins, terpenoids, alkaloids, glycoside, sterols, lignins quinones, and sponins using the previously reported methods[6-8] with some modifications. Control negative containing no *G. pedunculata* extract was performed in each test to ensure that the coloration change in the test was caused by *G. pedunculata* extract. Each test, along with negative control, was replicated three times.

2.4.1 Test for flavonoids

Shinoda test was used to detect for flavonoids. About 0.2 g of the extract was dissolved in 1 ml of ethanol, warmed and then filtered. Three pieces of magnesium ribbon was then added to the filtrate followed by few drops of concentrated hydrochloric acid. Presence of pink, orange, or red to purple coloration indicates the presence of flavonoids.

2.4.2 Test for tannins

Ferric chloride test was used to detect for tannins. About 0.2 g of the extract was stirred with about 1 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to the filtrate, occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

2.4.3 Test for terpenoids

Salkowski test was used to detect terpenoids. One milliliter of the extract was mixed with 1 ml of chloroform, and 0.5 ml of concentrated sulphuric acid was carefully added to form a layer. Reddish brown coloration of the interface indicates the presence of terpenoids.

2.4.4 Test for alkaloids

Dragendorff test was used to detect for alkaloids. Two milliliters of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. After adding 1 ml of Dragendroff's reagent (potassium bismuth iodide solution), presence of orange brown precipitate indicates the presence of alkaloids.

$FeCl_3$ test was also used to detect alkaloids. One drop of ferric chloride solution was added to the extract. Formation of yellow precipitate shows the presence of alkaloids.

2.4.5 Test for glycosides

Glycosides were detected by using Salkowski test. 2.0 ml of the concentrated sulphuric acid were added to the extract. Reddish brown color formed indicates the presence of steroidal aglycone part of the glycoside.

2.4.6 Test for sterols

Salkowski test was used to detect for sterols. Few drops of concentrated sulphuric acid were added to the extract in chloroform. Appearance of red color at the lower layer indicates the presence of sterols.

2.4.7 Tests for lignins

Lignins were detected by using Labat test. Gallic acid was added to the extract, the formation of olive green color shows the presence of flavonoids.

2.4.8 Tests for quinones

Alcoholic KOH test was used to test for quinones. When alcoholic KOH was added to the test samples, the appearance of red to blue color indicates the presence of quinones.

2.4.9 Test for Saponins

Foam test was used to detect for saponins. One gram of the extract was boiled with 5 ml of distilled water, filtered. To the filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 mins. Frothing which persisted on warming is taken as an evidence for the presence of saponins.

2.5 Determination of total phenolic content

The total phenolic content was determined with Folin-Ciocalteu method according to the method of Zhou, K. et al. with some modifications.[9] In brief, 0.1 ml of the extract (0.01 g/ml) or standard solution of gallic acid was mixed with 1.5 ml of 20% w/v sodium carbonate, 0.25 ml of Foline–Ciocalteu reagent and 1.15 ml of pure water, the mixture was left in the dark for 10 min at room

temperature. The absorbance was measured at 765 nm using a spectrophotometer (Jasco V-730 spectrophotometer, Japan). The analysis was performed in triplicate. The concentration of phenolic compounds was expressed as mg of gallic acid equivalents per gram of extract (mgGAE/g) by comparison with standard curve of gallic acid.

2.6 Determination of total flavonoid content

The total flavonoid content was determined according to the method of Ramamoorthy et.al with some modifications.[10] 0.15 ml of 2% $AlCl_3$ was mixed with 0.7 ml of the extract (0.01 g/ml) or standard solution of quercetin and 2.3 ml of pure water. The mixture was incubated for 10 min at room temperature. The absorbance was measured using a spectrophotometer (Jasco V-730 spectrophotometer, Japan) at 510 nm against a prepared reagent blank. The analysis was performed in triplicate. Total flavonoid content was expressed as quercetin equivalent per gram of the extract (mg QE/g).

2.7 Determination of antioxidant activity by DPPH radical scavenging assay

Free radical scavenging capacity of the extracts was determined by using the scavenging of the stable 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical according to the method of Mensor, L.L. et al. with some modifications.[11] One milliliter of each sample was added to 2 ml of DPPH in EtOH (0.1 mM). After incubation for 30 min in the dark, the absorbance of each sample was measured at 514 nm spectrophotometrically (Jasco V-730 spectrophotometer, Japan). The DPPH solution was used as a negative control. Gallic acid was used as the reference standard. Triplicate determinations were performed. The percentage of DPPH scavenging activity was calculated using the following formula:

$$\% \text{ antiradical activity} = [(A_c - A_s)/ A_c] \times 100$$

where A_c is the absorbance of the control and A_s is the absorbance of the sample. The IC_{50} values denote the concentration of the sample which is required to scavenge 50% of DPPH free radical

2.8 Statistical analysis

All work was done in triplicates and the data presented are means ± S.D. of three independent determinations. Significance was accepted at $p>0.05$.

3. Results & Discussion

3.1 Percentage yield of extracts

Previous research reports have shown that ethanol was the suitable solvent for the extraction of antioxidant compounds, including phenolic compounds by maceration method.[12] In addition, it was found that the extraction yield of ethanolic extracts was quite high due to the ability of ethanol to extract a wide range of compounds. Therefore, ethanol was chosen to use as the extraction solvent in this study. The percentage yield for the ethanolic extracts of *G. pedunculata* was found to be 24.25±2.77. The high extraction yield obtained may be described to the development of hydrogen bonds between polyphenols' hydroxyl groups and the electronegative oxygen of ethanol.[12]

3.2 Phytochemical screening

The phytochemical analysis of the extract of *G. pedunculata* is shown in the Table 1.

Table 1. Phytochemical screening of fruit extract of *G. pedunculata*.

Phytochemical tested	Test methods	Results
Flavonoids	Shinoda test	+
Tannins	Ferric chloride test	-
Terpenoids	Salkowski test	+
Alkaloids	Dragendorff test	-
	Ferric chloride test	-
Glycosides	Salkowski test	+
Sterols	Salkowski test	+
Lignins	Labat test	-
Quinones	Alcoholic KOH test	-
Saponins	Foam test	-

+ = Present, − = Absent

The results of phytochemical screening revealed the presence of various bioactive components such as flavonoids, terpenoids, glycosides and sterols. Each phytochemical showed potency towards some biological action. Flavonoids have antiviral, antibacterial, and antioxidant, properties. They also regulate gene expression and modulate enzymatic action.[13] Terpenoids have been reported to be useful in the prevention and therapy of several diseases, including cancer, and also to have antimicrobial, antiviral, antifungal, antiparasitic, antispasmodic, immunomodulatory, and anti-inflammatory properties. In addition, terpenoids are known to have insecticidal properties.[14-19] Cardiac glycosides have been used to treat congestive heart failure and cardiac arrhythmia.[20] Sterols derived from plants are known to have blood cholesterol-lowering effect via partial inhibition of intestinal cholesterol absorption. Other claimed benefits of phytosterols are possible anti-atherogenic effects, immune stimulating and anti-inflammatory activities. Besides, there is emerging evidence suggesting that particularly plant sterols may have beneficial effects against

the development of different types of cancers, like colorectal, breast and prostate cancers. [21-24]

3.3 Total phenolic and flavonoid contents

The total phenolic and flavonoid contents of the crude extract of *G. pedunculata* is presented in Table 2.

Table 2. Total phenolic acid and flavonoid contents of the extract of *G. pedunculata*.

Extracts	Total phenolic (mgGAE /g extract)	Total flavonoids (mgQE/g extract)
Fruit	74.37±6.31	175.90±0.40

Values are written as means±SD of three experiments

Phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity.[25] The hydroxyl groups in plant extracts are responsible for facilitating free radical scavenging. Phenolic content was determined using the Folin–Ciocalteu reagent. The results were derived from a calibration curve of gallic acid and expressed in gallic acid equivalents (GAE) per gram dry extract weight. The analysis of the fruit extracts of *G. pedunculata* showed the total phenolic content of 74.37±6.31 mgGAE/g extract.

Flavonoids are secondary metabolites with antioxidant activity, the potency of which depends on their molecular structures, the position of the hydroxyl group and other features in its chemical structure. They are abundantly found in plants as their glycoside.[26] The flavonoid contents in the plant extracts were determined using aluminium chloride in a colorimetric method. The results were derived from the calibration curve of quercetin (0.03–0.21 mg/ml) and expressed in quercetin equivalents (QE) per gram dry extract weight. The analysis of the fruit extracts of *G. pedunculata* showed the total flavonoid content of 175.90±0.40 mgQE/g.

3.4 Antioxidant activities

Antioxidant activities of the fruit extracts of *G. pedunculata* were assessed to identify potential sources of substances possibly useful against the effects of free radicals by DPPH method.

DPPH is a free radical, stable at room temperature, which produces a violet solution in ethanol. The DPPH assay is based on the ability of the stable DPPH free radical to react with hydrogen donors. The DPPH• radical displays an intense UV-VIS absorption spectrum. In this test, a solution of radical is decolorized after reduction with an antioxidant (AH) or a radical (R•) in accordance with the following scheme:

$$DPPH\bullet + AH \rightarrow DPPH\bullet\text{-}H + A\bullet,$$
$$DPPH\bullet + R\bullet \rightarrow DPPH\bullet\text{-}R.$$

The results of inhibition study are presented in Table 3. The results in Table 3 presents the half-maximal inhibitory concentration (IC_{50}) values and the equation formular.

Table 3. DPPH Inhibitory concentration (IC_{50}, µg/ml) of the extracts of *G. pedunculata*.

Extract	IC_{50}	Formula
Fruit	22.40±0.46	y = 1.9394x + 6.0940
Gallic acid	4.68±0.49	y = 9.6949x + 4.3471

Values are written as means±SD of three experiments

The fruit extract of *G. pedunculata* showed antioxidant potential with IC_{50} value of 22.40±0.46 µg/ml as compared to the standard gallic acid which possesses IC_{50} of 4.68±0.49 µg/ml. These indicated that the DPPH free radicals were scavenged by the fruit extracts of *G. pedunculata* and gallic acid with different capacities. Antioxidant potential is inversely proportional to inhibitory concentration (IC_{50}) value which was calculated from the linear regression of the percentage inhibition versus extract concentration. Hence, standard gallic acid showed higher antioxidant potential than the fruit extract of *G. pedunculata*. In addition, it was found that the DPPH free radical scavenging was antioxidant concentration dependent. Table 4 shows example data of this dependency characteristic. The percentage of antiradical activity of the fruit extracts was increased from 8.65±0.21 to 22.03±0.36.

Table 4. Antioxidant concentration dependent on DPPH free radical scavenging.

Extracts	**concentration (µg/mL)**	**%inhibition**
Fruit	1.67	8.65±0.21
	3.33	12.07±0.80
	5.00	15.24±1.26
	6.67	18.55±0.70
	8.33	22.03±0.36

4. Conclusion

The results of this study revealed that the ethanolic extract of *G. pedunculata* fruit is the primary antioxidants, which neutralizes free radicals and thus inhibits free radical-mediated reactions. For this reason, *G. pedunculata*, a healthy fruit commonly found in southern Thailand specifically in Phuket may help prevent or slow the progression of various oxidative stress-related diseases and can be used as an easily accessible source of natural antioxidant. However, further investigation on isolation, characterisation and identification of individual bioactive compounds responsible for bioactivities and possible synergy amongst them need to be studied and established.

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References

1. Phaniendra, A.; Jestadi, D.B.; Periyasam, L. *Indian J. Clin. Biochem.* **2015,** *30(1)*, 11–26.
2. Pisoschi, A.M.; Cheregi, M.C.; Danet, A.F. *Molecules* **2009**, *14*, 480 - 493.
3. De Fatima, A.; Modolo, L.V.; Conegero, L.S.; Pilli R.A.; Ferreira, C.V. *Curr. Med. Chem.* **2006,** *13*, 3371-3384.
4. Richards, A.J. Botan. J. Linn. Soc. **1990**, *103(3)*: 251-261.
5. Kagyung, R.; Gajurel, P.R.; Rethy, P.; Singh, B. *Indian J. Trad Knowl.* **2010**, *9*, 496- 501.
6. Trease, G.E.; Evans, W.C. *Trease and Evan's Textbook of Pharmacognosy*, 13th ed.; Cambridge University Press, 1989.
7. Alamzeb, M.; Khan, M.R.; Ali, S.; Shah, S.Q.; Mamoon, U.R. *Bangladesh J. Pharmacol.*, **2013**, *8(2)*, 107-109.
8. Rajinikanth, M.; Samatha, T.; Srinivas, P.; Shyamsundarachary, R., Rama, S.N. *Int. J. Pharm. Sci. Rev. Res.*, **2013,** *20(1)*, 134-139.
9. Zhou, K.; Yu, L. *LWT* **2006**, *39*, 1155–1162.
10. Ramamoorthy, P.K.; Bono, A. *J. Eng. Sci. Technol*. **2007**, *2(1)*, 70-80.
11. Mensor, L.L.; Menezes, F.S.; Leitao, G.G.; Reis, A.S.; Santos, T. S.; Coube, C.S. *Phytother. Res.* **2001,** *15*, 127–130.
12. António, J.Q.; Mota, F.L.; Pinho, S.P.; Macedo, E.A. *J. Phys. Chem. B* **2009**, *113(11*), 3469–3476.
13. Aleksandra, K.; Dorota, S.W. *Rocz Panstw Zakl Hig*. **2014**, *65(2)*, 79-85.
14. Roslin, J.T., Anupam, B. *World J. Hepatol*. **2011**, *3(9*), 228-249.
15. Rabi, T.; Bishayee, A. *Breast Cancer Res Treat*. **2009**, *115*, 223-239.
16. Wagner, K.H.; Elmadfa, I. *Ann Nutr Metab*. **2003**, *47*, 95-106.
17. Sultana, N.; Ata, A. *J. Enzyme Inhib. Med. Chem.* **2008**, *23*, 739-756.
18. Shah, B.A.; Qazi, G.N.; Taneja, S.C. *Nat Prod Rep*. **2009**, *26*, 72-89.
19. Theis, N.; Lerdau, M. *Int J Plant Sci*. **2003**, *164*, S93-S103.
20. Kren, V.; Martínková, L. *Current Medicinal Chemistry* **2001**, *8(11*), 1303-1328.
21. Bradford, P.G.; Awad, A.B. *Mol Nutr Food Res*. **2007**, *51*, 161-170.
22. Bouic, P.J. *Drug Discov Today*. **2002**, *7*, 775-778.
23. Jones, P.J.; Ntanios, F.Y. Raeini-Sarjaz, M.; VANSTONE, C.A. *Am J Clin Nutr*. **1999**, *69*, 1144-1150.
24. Navarro, A.; De Las Heras B.; Villar A. *Clem. Biol Pharm Bull*. **2001**, *24*, 470-473.
25. Soobrattee, M.A.; Neergheen, V.S.; Luximon-Ramma, A.; Aruoma, O.I.; Bahorun, T. *Mutat. Res.-Fund. Mol. Mutagen.* **2005**, *579*, 200–213.
26. Rajanandh, M.G.; Kavitha, J. *Int. J. Pharm Tech Res.* **2010,** *2*, 1409-1414.