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INTRODUCTION

Momordica cochinchinensis of Cucurbitaceae family has been known as "Fhuck khow" in Thailand and called "GAC" in Vietnam. It has been consumed as indigenous vegetable and fruit. It was found that peels and seed membranes of GAC fruit compose of high levels of β-carotene, α-tocopherol and lycopene [1]. Seed membrane of ripe GAC fruit (orange color) was reported to contain high levels of β-carotene and lycopene at about 10 and 30 times greater than in carrots and tomatoes [2], respectively. Though GAC is increasing in popularity, very few information of its anti-oxidant activity was reported. Since traditional solvent extraction has some disadvantages i.e. some residual solvent left in the extracts and matrix, and there is always some level of environmental contamination from their use [3]. Therefore, in this study GAC (seed and peel) extracts were prepared by using supercritical fluid carbon dioxide (SCF-CO_2) method. Carrots and tomatoes were also extracted in similar manner and used as reference samples. The phytochemical components of SCF-CO_2-GAC peel (GAC-P) and seed membrane (GAC-SM) extracts were determined by three analytical parameters including phenolic, carotenoid and flavonoid contents. The anti-oxidant capacity of these GAC-P and GAC-SM extracts was assessed on superoxide ($O_2^{\bullet}$) and hydroxyl ($OH^{\bullet}$) radicals scavenging activity using photochemiluminescence (PCL) and deoxyribose (DR) assays, respectively. Their cytotoxic property was evaluated by the MTT assay on TK6 human lymphoblast and L929 mouse fibroblast cell lines.

MATERIALS AND METHODS

Preparation of supercritical fluid carbon dioxide (SCF-CO_2) extraction The ripe fruits of GAC were purchased from Nakhonpathom province of Thailand (Figure 1). They were washed to remove any contaminants. The peels (P) and seed membranes (SM) were separated and dried at 50°C in hot air oven for 48 h. Dried samples were ground and stored in the refrigerator (4°C) in light-protective container. Five hundred grams (500 g) of air-dried peel or seed membrane were subjected to supercritical fluid CO_2 extraction (SCF-CO_2) under suitable conditions (data not shown regarding patent procedure). Carrots and tomatoes were prepared and extracted by the similar manner and used as reference samples.

Phytochemical component determination

Total phenolic content (TPC) The total phenolic content of GAC-P, GAC-SM, carrot and tomato extracts was determined using the Folin-Ciocalteu's method [4] with a slight modification due to soluble capacity of extracts. Gallic acid was used as the standard phenolic. The results were expressed in term of mg gallic acid equivalents (GAE)/mg sample extract.

Total flavonoid content (TFC) The total flavonoid content of GAC-P, GAC-SM, carrot and tomato extracts was determined using the aluminum chloride colorimetric method [5]. Rutin was used as the standard flavonoid. The results were expressed in term of mg rutin equivalents (RE)/mg sample extract.

Total carotenoid content (TCC) The total carotenoid content of GAC-P, GAC-SM, carrot and tomato extracts was determined using a spectrophotometer. β-carotene was used as the standard carotenoid. The results were expressed in term of β-carotene equivalent (BE)/100 mg sample extract.

Antioxidant capacity determination

Superoxide anion radical ($O_2^{\bullet}$) scavenging activity by PCL assay The anti-oxidant activity of GAC-P, GAC-SM, carrots and tomatoes was determined using photochemiluminescence (PCL) [6-7] method by Photochem® (Analytik Jena, Germany). Its principle is based on the photo-induced auto-oxidation inhibition of luminol by antioxidants mediated from the superoxide anion ($O_2^{\bullet}$) radicals. The antioxidant evaluation was performed for both water (ACW) and lipid (ACL) soluble substance systems using the

ascorbic acid and trolox as standard antioxidants. Antioxidant activity would be indicated by $O_2^{-\bullet}$ reduction in the presence of test samples compared with the standard antioxidant (construction of a calibration curve with Trolox for antioxidant capacity of ACL or ascorbic for antioxidant capacity of ACW). The sensitivity of the photochemiluminescence (PCL) assay lies within nmol quantities of substances. For antioxidant determination, each GAC SCF-CO_2 sample was prepared by dissolving 10 mg in 1 mL of dimethyl sulfoxide (DMSO) and filtered through 0.45 mm syringe filter. All samples were determined in triplicate.

Hydroxyl radical ($OH^{\bullet}$) scavenging activity by 2-deoxyribose (2-DR) degradation assay The hydroxyl radical ($OH^{\bullet}$) scavenging activity of GAC, carrots and tomatoes was assessed using the *2*-deoxyribose (2-DR) degradation *assay* (Genaro-Mattos, 2009). The 2-DR method is based on the determination of malondialdehyde (MDA) pink chromogen which was a degraded product of 2-deoxyribose (2-DR) damaged by $OH^{\bullet}$. Each sample fractions were prepared as previously mentioned in PCL assay except using distilled water as solvent. Typical reactions were started by the addition of 50 µM $FeCl_3$ to solutions (0.5 mL final volume) containing 5 mM 2-DR, 100 µM EDTA, 10 mM phosphate buffer (pH 7.2), 0.5 mM H_2O_2 and various concentration of sample fractions in presence of 100 µM ascorbic acid (reducing agent) for starting the reaction and generated $OH^{\bullet}$. Reactions were carried out for 10 min at room temperature and stopped by the addition of 0.5 mL 2.8% trichloroacetic acid (TCA) followed by the addition of 0.5 mL thiobarbituric acid (TBA) solution. After boiling for 15 min, solutions were allowed to cool at room temperature. The absorbance of reaction mixture was measured to determine MDA pink chromogen at 532 nm in micro-plate reader system (GENios Plus, TECAN®, Australia). All samples were tested in triplicate.

Cytotoxicity determination by MTT assay Cytotoxic property of GAC-P and GAC-SM extracts was performed on 2 cell lines including the TK6 human lymphoblasts and L929 mouse fibroblasts using the MTT assay. The principle of MTT is based upon the quantitative colorimetric method for determining cell proliferation. The MTT (3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium-bromide) is a yellow water soluble tetrazolium dye that is reduced by mitochondrial dehydrogenase, in living but not dead cells, to a purple formazan product that is insoluble in aqueous solutions. The amount of MTT-formazan formation can be determined spectrophotometrically after being solubilized in a suitable solvent such as dimethylsulfoxide (DMSO). Surviving cell numbers (living cells) are determined indirectly by MTT dye reduction. Both cell lines were exposed to GAC-P and GAC-SM at different concentrations ranging from 1,000 to 5000 µg/ml for 4 h (short exposure) and 24 h (long exposure). Following the exposure times, cell viabilities of TK6 and L929 were determined and reported as the IC_{50}, which refers to the concentration of test sample that inhibits cell growth by 50%.

$$\% \text{ Viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of untreated cells}} \times 100$$

RESULTS AND DISCUSSION

Phytochemical component determination

Total phenolic content (TPC) A summary of total phenolic content of extracts of GAC-P, GAC-SM, tomatoes and carrots was demonstrated in **Table 1.** Among these 4 extract samples, it was found that carrot exhibited the highest phenolic content at 0.0261 ±0.010 mg GAE/mg extract.

Table 1 Values of total phenolic content (TPC)

Samples	Total phenolic content (mg GAE/mg extract)
GAC seed membrane (GAC-SD)	0.0156 ± 0.039
GAC peel (GAC-P)	0.0156 ± 0.039
Tomato (T)	0.0261 ± 0.010
Carrot (C)	0.0160 ± 0.032

Results were expressed as mean ± SD (n = 3).

Total flavonoid content (TFC) A summary of total flavonoid content of four extracts including GAC-P, GAC-SM, tomatoes and carrots was shown in **Table 2.** The highest flavonoid content (0.167 ± 0.005 mg RE/mg extract) was observed in peel extract of GAC (GAC-P) sample.

Table 2 Values of total flavonoid content (TFC)

Samples	Total flavonoid content (mg RE/mg extract)
GAC seed membrane (GAC-SD)	0.0902 ± 0.023
GAC peel (GAC-P)	0.1670 ± 0.005
Tomato (T)	0.1015 ± 0.003
Carrot (C)	0.0958 ± 0.002

Results were expressed as mean ± SD (n = 3).

Total carotenoid content (TCC) The total carotenoid content was summarized in **Table 3**. Of this assay, β-carotene was used as the standard carotenoid. The results were expressed in term of β-carotene equivalent (BE)/100 mg sample extract. The result showed the highest carotenoid content value (483.12 µg ± 0.0005 µg BE/100 mg extract) was detected in an extract of GAC peel (GAC-P).

Table 3 Determination of total carotenoid content (TCT)

Samples	**Total carotenoid content (µg BE/ 100 mg extract)**
GAC seed membrane (GAC-SD)	42.1 ± 0.008
GAC peel (GAC-P)	483.12 ± 0.0005
Tomato (T)	354.67 ± 0.004
Carrot (C)	1.66 ± 0.016

Results were expressed as mean ± SD (n = 3).

Superoxide anion radical ($O_2^{-\bullet}$) scavenging activity The highest antioxidant activity was found in GAC-P for both the ACL and ACW measurement system at 1.873±0.034 nmol Trolox equiv/100 µg and at 0.166 ±0.007 nmol Ascorbic acid equiv/100 µg, respectively. The tomato sample (ACL = 1.144±0.109 nmol Trolox equiv/100 µg, ACW = 0.131±0.053 nmol Ascorbic acid equiv/100 µg) exhibited a greater antioxidant activity over carrot (ACL = 0.412±0.099 nmol Trolox equiv/100 µg, ACW = 0.062±0.015 nmol Ascorbic acid equiv/100 µg). The PCL results also indicated that GAC-P possessed a higher $O_2^{-\bullet}$ scavenging activity than GAC-SM. The results of ACL and ACW suggested that the $O_2^{-\bullet}$ scavenger activity in SFE-P found both polar and non-polar phytochemical groups and the non-polar group was a more effective antioxidant than the polar group.

Hydroxyl radical ($OH^{\bullet}$) scavenging activity As demonstrated in **Figure 1**, the $OH^{\bullet}$ scavenging activity of GAC extracts exhibited a wide range of $OH^{\bullet}$ scavenging activity, proved from 8.14 ± 0.31 to 55.05 ± 1.09 in the % inhibition of the 2-DR degradation. The GAC-P extract was a more effective inhibitor of the $OH^{\bullet}$ by exhibiting (55.05 ± 1.09%) than SFE-SM sample (8.14 ± 0.31%) for the inhibition of 2-DR degradation. However, it was observed that tomato (T) extract exhibited greater antioxidant activity over GAC-P and GAC-SM extracts in this assay. The wide range of % inhibition values among extracts was possibly caused by their solubility in water, which was the main solvent used in the deoxyribose assay.

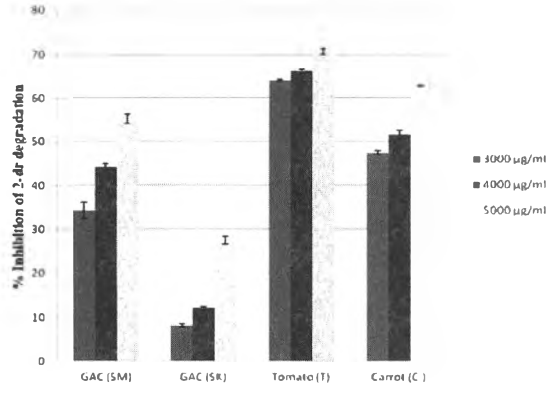

Figure 1 Inhibitory effect (%) of a 2-deoxyribose degradation of GAC-SM, GAC-P, tomato (T) and carrot (C) extracts. Results were expressed as a means ± SD (*n*=3).

Cytotoxic activity The MTT assay was carried out to examine the cytotoxicity of GAC-P and GAC-SM in two different cell lines including TK6 human lymphoblasts and L929 mouse fibroblasts. Results of MTT assay of L929 and TK6 displayed in **Table 4.** The MTT results revealed cell survival rates greater than 80% and their IC_{50} > 1,000 µg/ml indicating a non-cytotoxic property of GAC peel and seed membrane extracts.

Table 4. The growth inhibitory effect-IC_{50} values (µg/ml) of GAC-SM and GAC-P extracts on the TK6 and L929 cell lines by MTT assay after 4 and 24 h exposuretime, respectively.

Cell line	Extract	Treatment time (h)	IC_{50} (ug/ml)
TK6	GAC-SM	4	>5,000
		24	>5,000
	GAC-P	4	3,512
		24	1,979
L929	GAC-SM	4	>5,000
		24	>5,000
	GAC-P	4	>5,000
		24	3,162

CONCLUSION

The present study firstly revealed that *Momordica cochichinensis* or GAC fruit peel (SCF-P) and seed membrane (SCF-SM) extracts displayed low to non-cytotoxic to TK6 and L929 cells, as determined by MTT assay. In addition, the peel extract possessed higher $O_2^{\cdot-}$ and $OH^{\cdot}$ scavenging activity than seed membrane extract. For the health promoting effect, increased consumption of *M cochichinensis* antioxidants in the diet of individuals is strongly recommended. Upon this purpose, further biochemical studies may be needed to characterize its antioxidant defense characteristics or counteracting the effects of any pro-oxidant.

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