ANALYSIS OF TOTAL PHENOLIC COMPOUND AND INHIBITION POWER IN EXTRACTED SUBSTANCE FROM KAI ALGAE (*Cladophoraspp*)

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ABSTRACT

Kai algae is the popular freshwater algae for rural people in the north of Thailand. The aim of this work was to evaluate the efficiency of crude extracted from two types of algae as fresh Kai algae and dried Kai algae by analysis total phenolic compound content and antioxidising inhibition power. The extracted substances were prepared by 5 methods such as boiling with water ,extraction with sodium hydroxide , alcoholic extraction , acid extraction and mechanical extraction, finally all extracted substances were analysed total phenolic content and antioxidising inhibition power. The results showed that dried Kai algae which extracted by 0.3 N sodium hydroxide at 60 ^{o}C gave the best extracted substance,since its contained highest total phenolic substance approximately 1066.96 ± 15.12 mg GAE/100 g. All extracted substance showed the antioxidising inhibition power as 57-88 %. The extracted substance from Kai algae showed the potential as source of antioxidant compound.

Keywords: Extracted substance, Kai algae,*Cladophora spp*., Total phenolic compound, inhibition power.

INTRODUCTION

In general algae is an important source for various bioactive compounds example as antimicrobials, antivirals and antioxidant properties (Pulz.O&Gross.W, 2004). Kai algae is one type of green freshwater algae ,it general grow up in clean natural river such as Mekhong river and Nan river(Fahprathanchai.P,et al., 2006). The scientific name of this algae is known as*Cladophora* spp**.***(Cladophoraglomerata*Kutzing).Kai algaeshows an important medicinal properties such as anti-inflammatory, cure Gastric Ulcer, reduce thirsty, antidiabetic, renoprotective (Srimaroeng.C.A., et al., 2015), and good for dark hair(Peerapornpisal.Y.,et al., 2006). The algae also contains many important nutrients such as protein ,carbohydrate ,vitamin and mineral,(Peerapornpisal.Y, et al.,2006), so rural people in the Northern part of Thailand use it as food. There have many food products that processed from this algae such as Kai gee , Kai dried snack. There was no report about the other products like the other popular algae as *Spirulina* . Spirulina algae is also a new economic plant in Thailand , it is also cultivated for sold as a dietary supplement(Belay.A, 2008) to serve good health for human.

Eventhough ,Kai algae has a good properties as referred , but it was not research report about the potential of algae to be one additive in some cosmetics as moisture cream.From the preliminary test about total phenolic compound content (Luangsuwan.R&Chulalaksananukul.W, 2013), it only an idea to get an important nutrient from this algae.It has been known that many plants such as green tea , grape, medicinal herb plants contains various components with anti-oxidative properties , some may showed anti carcinogenic properties (Arce.L, et al.1998). Many researchers report that those anti-oxidative plants could be applied for the many industries. An interesting industry as cosmetic industry is the one that used many substances which contain an important property such as

antioxidant property. The growth in cosmetic and drug industries trend to continuous increase, so the aim of this work was to analysis the total phenolic content and antioxidising inhibition properties in extracted substance from Kai algae for estimate the potency of fresh algae to be the additive in moisture cream.

MATERIALS AND METHOD

All chemical reagents used in this work were an AR grade, purchased from Fluka and Merck**.** Allfresh Kai algae and dried Kai algae got from Nan River in Thawangpha district of Nan Province (Northern part of Thailand).

Part1. Preparation Method of Crude Extracted

Method1: Each type of Kai algae 5.0000 g was extracted by boiling in distilled water and varied heating temperature at 40 ^{o}C , 60^{o}C and 80 ^{o}C for 4 hours.The residue algae was filtered out and collected filtrate.The filtrate of each extracted solution was analysed as part 2

Method2. Each type of Kai algae 5.0000 g was extracted by sodium hydroxide 0.1, 0.3 and 0.5 N and heated in water bath (Memmert) by variation of the heating temperature at 40 ^{o}C , 60^{o}C and 80 ^{o}C for 4 hours.After filtration of residue algae ,the filtrate of each extracted solution was collected and analysed as in part 2

Method3. Each type of Kai algae 5.0000 g was placed into 200 ml of ethanol and left at room temperature for 48 hours. The filtrate of each extracted solution was also analysed as in part 2

Method4. Each type of Kai algae 5.0000 g was soaked in 200 ml of 2% HCl in methanol for 48 hours. The filtrate of each extracted solution was analysed as in part 2

Method5. Each type of Kai algae 5.0000 g was pressed and crushed and filtered the residue algae out. Then the filtrate of each extracted solution was collected and analysed as part 2.

Part2.Analysis Method
Analysis of Total Phenolic Compound

The analysis method modified from Arce.L,et al. (1998) ,the clear filtrate from part 2.1 were pipetted 0.4 ml and mixed with 2 ml of 10 % FolinCiocalteau reagent and 1.6 ml of 7.5 % Na_2CO_3 and kept at room temperature for 30 mins. The mixing solution was measured an absorbance at 765 nm by Ultraviolet Visible Spectrophotometer(UV -VIS Shimadzu Model UV100) and calculated the Total phenolic compound content as gallic acid equivalent(GAE).

Analysis of Antioxidant Inhibition Power

The analysis method modified from Arce.L,et al. (1998),the clear filtrate from part 2 were pipetted 600 µL and mixed with 600 µL of 0.1 mM DPPH then measured an absorbance of the complex color from the reaction at 517 nm. The inhibition power was calculated by comparing with standard BHT.

Statistical Analysis

All determinations were carried out at least in five replication and values were averaged. The results are expressed as mean ± S.D. values; statistical differences between means were determined by one way ANOVA.

RESULTS AND DISCUSSION

After extraction the crude extracted substance by the method1 and analysed the total phenolic compound content followed by part 2 the result showed as in table 1.

Table 1.Total phenolic contents in crude extracted substances from Kai algae by heating in water at 40,60 and 80 ^{o}C

Heating Temperature(^{o}C)	Type of Kai algae	Total phenolic compound content (mg GAE/100 g)
40	Fresh algae	5.02 ± 0.01
40	Dried algae	320.59 ± 8.95
60	Fresh algae	6.86 ± 0.02
60	Dried algae	372.99 ± 7.86
80	Fresh algae	7.92 ± 0.05
80	Dried algae	385.85 ± 5.44

Note: The 5 Kai algae samples were used in each extraction.

From table1 presented that the Total phenolic content in both fresh Kai algae and dried Kai algae trend to increase as increasing heating temperature of water as extracted solvent. However, the crudeextracted substances by all conditions from dried Kai algae showed the higher Total phenolic content than in fresh Kai algae. Since, the dried Kai algae lost some moisture content from cellulose fiber of Kai algae body, so all important substances may be concentrated in dried fiber.The result from the variation of heating time in extraction process by the method 1 was also studied as showed the Total phenolic contents as in table 2.

Table 2.Total phenolic contents in crude extracted substances from Kai algae using heating water at 80 ^{o}C for 0 – 4 hours

Heating Times (hrs)	Type of Kai algae	Total phenolic compound content (mg GAE/100 g)
0	Fresh algae	7.92 ± 0.05
0	Dried algae	372.99 ± 7.86
2	Fresh algae	45.23 ± 0.02
2	Dried algae	576.50 ± 20.59
4	Fresh algae	50.80 ± 5.04
4	Dried algae	422.99 ± 10.34

Note: The 5 Kai algae samples were used in each extraction.

The result showed the dried algae that was extracted by hot water at 80 ^{o}C for 2 hours could extracted the maximum quantity of an important substance , an importance substance in Kai algae were solvated and leaked from the cell of plant easier than using low temperature. The dried algae which was treated with hot water at 80 ^{o}C for 2 hours was the best condition of method 1. In the method 2 , all Kai algae were extracted with 0.1 - 0.5 N of sodium hydroxide solution and the result presented as in table 3.

Table 3.Total phenolic contents in crude extracted substances from Kai algae using sodium hydroxide at 0.1-0.5M

Sodium hydroxide concentration (N)	Type of Kai algae	Total phenolic compound content(mg GAE/100 g)
0.1	Fresh algae	77.51 ± 0.66
0.1	Dried algae	531.26 ± 6.86
0.3	Fresh algae	93.47 ± 2.49
0.3	Dried algae	916.04 ± 35.46
0.5	Fresh algae	80.46 ± 1.74
0.5	Dried algae	650.69 ± 17.09

Note: The 5 Kai algae samples were used in each extraction.

The extraction Kai algae with 0.3 N sodium hydroxide showed the highest content of total phenolic compound from algae,so this condition showed the highest efficiency for extract the important substance. Thus, the variation of heating time between 40 to 80^{o}C for using in extraction system by 0.3 N sodium hydroxide were also studied as showed the result in table 4.

Table 4.Total phenolic contents in crude extracted substances from Kai algae using 0.3N sodium hydroxide with variation on heating temperature

Heating Temperature(^{o}C)	Type of Kai algae	Total phenolic compound content (mg GAE/100 g)
40	Fresh algae	80.46 ± 1.74
40	Dried algae	650.69 ± 17.09
60	Fresh algae	208.96 ± 5.02
60	Dried algae	1066.96 ± 15.12
80	Fresh algae	159.45 ± 1.86
80	Dried algae	968.34 ± 16.53

Note: The 5 Kai algae samples were used in each extraction.

At 60 ^{o}C of heating temperature, the extracted substances from both type of Kai algae contained the highest Total phenolic content, it may concern that the base medium could destroy the cellulose fiber of plant better than using low temperature as 40^{o}C support on the work of Li.H,et al., (2007). However, heating process at high condition at 80 ^{o}C may destroy some part of phenolic compound in plant, since some substance such as β-carotene in plant(Wang.T.R.,et al. 2009)The five method of extractions processes from Kai algae showed the Total phenolic contents in all crude extracted as in table 5.

Table 5. Total phenolic compound content in crude extracted substance by 5 methods

Method	Total phenolic content (mg GAE/100g)	
	Fresh Kai algae	Dried Kai algae
1	0.02 ± 45.23	20.59 ± 576.50
2	5.02 ± 208.96	15.12± 1066.96
3	0.15 ± 14.33	2.55 ± 48.52
4	0.23 ± 76.56	2.88 ± 145.25
5	0.18 ± 7.33	1.74 ± 194.62

Note: The 5 Kai algae samples were used in each extraction.

The crude extracted substance from dried Kai algae which was treated as the method 2 showed the highest total phenolic content approximately 1067 mg GAE/100 g. However the crude extracted substance from all methods were analysed antioxidant inhibition power followed the part 2 as showed in figure1.

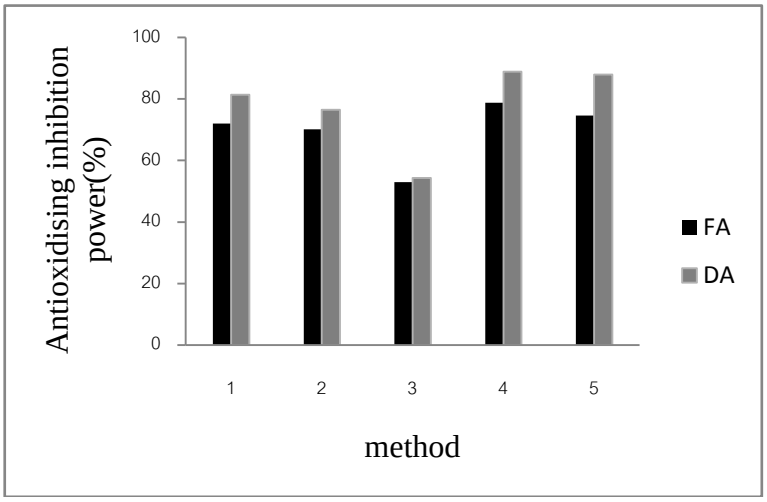

Figure1. Antioxidant inhibition power in Extracted substances form Kai algae samples

Note: FA = fresh algae DA = dried algae

The crude extracted substance by all methods showed the antioxidant inhibition power in the range of 57-88 %.The method 4 gave the extracted substance that contained highest antioxidant inhibition power. The antioxidant inhibition power did not relate with the total phenolic content like as referred by (Wang.T.R.,et al. 2009). Because of, there are many phenolic compounds in plant, some phenolic compound such as gallicacid ,ellagic acid(Amakura.Y, M.et al.,2000)have a small size in structure. The polar molecule that can dissolve in water , basic solvent as sodium hydroxide and polar solvent better than other solvent. Some phenolic compound that its nature has macromolecular structure with nonpolar structure such as some benzoic derivatives , p-anisic acid, they dissolve in non polar solvent better than in water (Natella.F,M.et al.,1999).

CONCLUSIONS

This work showed that crude extracted substance that extracted by each method showed the potential to be an antioxidant substance and could be use as the part of raw material use in cosmetic industry. All crude extracted substances have the efficiency to be antioxidant source of total phenolic compounds could be use in cosmetic industry. However, crude extracted from Kai algae using the method 2 gave the highest Total phenolic content as 1066.96 ± 15.12 mg GAE/100g.Thus, if the extracted substance must be use as additive; the crude extracted solution should be prepared by using 0.3N sodium hydroxide as an extracting solvent and heated at 60^{o}C for4hours.

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