ประสิทธิภาพการป้องกันแสงและฤทธิ์ต้านอนุมูลอิสระของสารสกัดฝอยทอง
Photo protective and Antioxidant Activity of *Cuscuta chinensis* Lam.

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บทคัดย่อ

สารสกัดหยาบฝอยทองได้ถูกนำมาวิเคราะห์ความสามารถในการป้องกันแสงแดดและการด้านอนุมูลอิสระ ซึ่งพืชฝอยทองได้มาจากอำเภอเมือง จังหวัดลำปาง โดยการศึกษานี้มีจุดประสงค์เพื่อจะศึกษาประสิทธิภาพสารกันแดดจากวัชพืช จากการศึกษาพบว่าสารสกัดฝอยทองมีฤทธิ์ต้านอนุมูลอิสระมากไปกว่านั้น สารสกัดฝอยทองยังมีความสามารถในการป้องกันแดดได้ดีอีกด้วย จากการทดลองจึงมีความเป็นไปได้ในข้อสรุปที่จะนำสารสกัดฝอยทองมาผลิตในเครื่องสำอางประเภทปกป้องรังสีจากแสงแดดได้

คำสำคัญ: สารสกัดฝอยทอง / สารกันแดด / สารต้านอนุมูลอิสระ / FRAP

Abstract

The crude extracts of *Cuscuta chinensis* (*C. chinensis*), was analyzed for photoprotective and antioxidant activity. The plant material is obtained from Lampang district, Thailand. The objective of this study was determine the effectiveness in photoprotective ability. *C. chinensis* had antioxidant activity. Moreover, it has ability in photoprotection. In addition, application of the crude extract of *C. chinensis* can be used in the formulation of cosmetic. In summary, the extract of Cuscuta chinensis Lam.can possible to be sunscreen agent in cosmetic product.

Keywords: Cuscuta chinensis / sunscreen / antioxidant / FRAP

Statement and significance of problem

Cuscuta chinensis Lam. (*C. chinensis*), is parasitic plants which lives with others plant can cause lack of water, minerals, and photosynthesis from the host. And it can easily reproduce and fast, these resources are unlimited for parasitic weeds unless the host is killed in the end[1] *C. chinensis* has found its use as a traditional medicine in China, Korea, Pakistan, Vietnam, India and Thailand. It is commonly used as an anti-aging agent, anti-inflammatory agent, pain reliever and aphrodisiac.[2] *C.*

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chinensis is Chinese herb which employed in traditional Chinese medicine have been used for centuries in the practice of medicated diet and dietetic therapy. The seeds of ***C. chinensis*** is a traditional Chinese medicine that is commonly used to nourish and improve the liver and kidney conditions in China and other Asian countries.[3] and immunostimulatory activity.[4] In addition, *C. chinensis* has been demonstrated to exert anti-aging effects and enhance memory by inducing the PC12 cells differentiation.[5] *C. chinensis* contains kaempferol and quercetin The family of plant compounds to which kaempferol and quercetin belong is known as the Flavonoid.[6] In addition, *C. chinensis* can live in summer and has character of heat resistance because it contains secoundary metabolite such kaempferol, quercetin and other compounds.[7] Therefore, the kaempferol, quercetin and other compounds of *C. chinensis* will be extracted and studied for the photoprotective and antioxidant activity. Moreover, this study will give basis information for development the crude extracted as a new sunscreen agent in *C. chinensis* to adapt for using a natural active ingredient in cosmetic and health care product.[8]

Figure 1. *Cuscuta chinensis* Lam.

Objectives

1. To study the antioxidant activity of *C. chinensis* crude extract
2. Study the UVA and UVB absorotion efficacy of *C. chinensis* crude extract

Materials and methods

Plant extraction: Preparation of plant material.[6]

C. chinensis was collected from Hangchat, Lampang in September, afternoon. Fresh plant of *C. chinensis* was washed and then kept in oven at 60-80 °C for 24 hours under <5 % humidity control. Then, Sample was finely grinded until become powder completely. Three liters of 95% ethanol was added into one kilogram of *C. chinensis* powder and kept at room temperature for 72 hours under

incubator shaker. The solution has been filtered to discard precipitant with white thin cloth at first and then filtered again by filter paper under Bucher and Vacuum. The filtrate was concentrated by vacuum rotary evaporator at 70 ℃. The retain water content of crude sample from evaporation was removed by freeze dryer for 36 hours. Then, the final sample crude extract was weighed and kept in freezer at 2 ℃

Anti-aging related to antioxidant activities assay: Determination of antioxidant power by Ferric Reducing Antioxidant Power (FRAP) assay.[9]

Prepare FRAP reagent by mixing 200 ml acetate buffer pH 3.6,20 ml TPTZ solution (2,3,5-triphenyltetrazol-2-ium chloride), 20 ml FeCl, solution and 24 ml distilled water. And Prepare 1 mM ferrous Sulphate as stanrd reagent in FRAP assay. *C. chinensis* extract is prepared at different dilutions (2, 5, 10, 20 and 40 ppm in ethanol). 6 ul of the tested sample was mixed with 200 ul of FRAP solution in micro-plate. The absorbance reading at 539 nm is taken after standing for 30 min at 37 ℃. The positive result shows the change of FRAP reagent from colorless solution into blue solution. Ethanol is used as a negative in this experiment. FRAP value of tested substance is determined by calculate from a standard linear regression of Ferrous Sulphate.

Determination of sun protection efficacy: Determination of Ultraviolet ray absorption on the UV region by UV-Vis spectrophotometer [10]

Crude extract of *C. chinensis* was weighted and dissolved in ethanol at vary concentration 0.125, 0.25, 0.50, 1.00, 2.00 and 4.00 %w/w. The solution is shacked until the crude extract dissolves completely. Tested substance is inserted in quartz cuvette and scanned in UV- Vis spectrophotometer under wave scan 200-400 nm with 750 nm/min scan speed. The UV ray absorption on the UV region of *C. chinensis* extract was determined by observe spectra graph between absorbance value and UV region wavelength. The positive control is Octyl Dimethyl PABA and the negative control is solvent used.

Conclusions

Total yield of *C. chinensis*

The *C. chinensis* crude extracts and percent yields from fresh aerial part of *C. chinensis* from maceration extraction with 95 % ethanol have been shown in Table 1.

Table 1 Total yield of *C. chinensis* extract in each experiment.

Experiment	Total yield of *C chinensis* Lam.			% yield (w/w)
	Fresh (g)	Air Dry (g)	Total Extract (g)	
1	10,000	1,000	31.32	0.3132
2	10,000	1,000	32.25	0.3225

Experiment	Total yield of *C chinensis* Lam.			% yield (w/w)
	Fresh (g)	Air Dry (g)	Total Extract (g)	
Total	20,000	2,000	63.53	0.3179

Determination of antioxidant activity by Ferric Reducing Antioxidant Power assay

The Optical Density (O.D.) of Ferrous sulfate at vary concentrations are shown in Figure **2.** A linear regression for the Ferrous sulfate between the O.D. against concentration. The correlation value in linear regression is significant (R-0.995). The O.D. of *C. chinensis* extract at vary concentrations in FRAP. A linear regression for the tested substance in FRAP is shown in Figure 2. The correlation value in linear regression is significant (0.997). The *C. chinensis* extract equivalent to $FeSO_4$ at each concentration. A linear regression between its concentration and FRAP. The correlation value in this linear regression is significant (0.998).

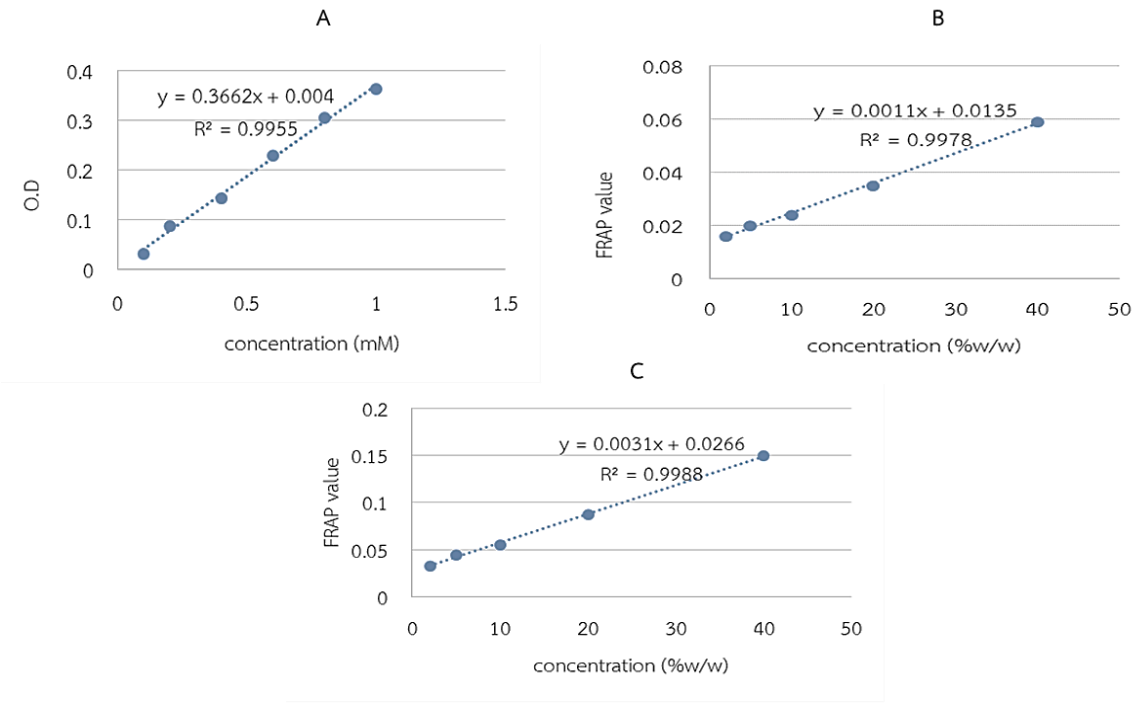

Figure 2. A linear regression of **A.** Ferrous sulfate in FRAP antioxidant assay. **B.** *C. chinensis* extract in FRAP antioxidant assay. **C.** FRAP value *C. chinensis* extract in different concentration that equivalent to $FeSO_4$

Determination of sun protection efficacy

Determination of Ultraviolet ray absorption on the UV region by UV-Vis spectrophotometer

The ability in UV absorption of ethanol (Negative standard) is shown in Figure 3. Absorbance values of ethanol is less than 2 at the wavelength 210 -350 nm. At the wave length 350-450 nm, ethanol cannot absorb UV ray in this region.

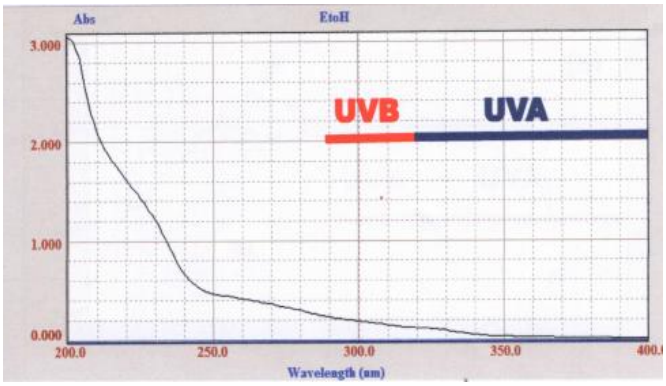

Figure 3. The UV absorbance spectra of ethanol

The ability in UV absorbance of different concentrations of crude extracts are shown in Figure 4-5. Different concentrations of crude extract (0.125, 0.25, 0.50, 1.00, 2.00 and 4.00 % w/w) show different spectra graph.

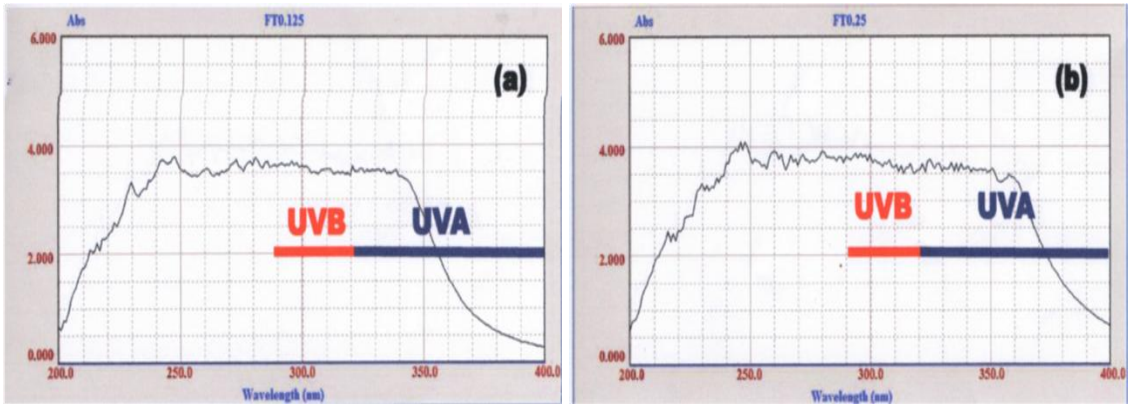

Figure 4. The UV absorbance spectra of *C. chinensis* extract in different concentrations **(a)** 0.125%w/w. **(b)** 0.25%w/w.

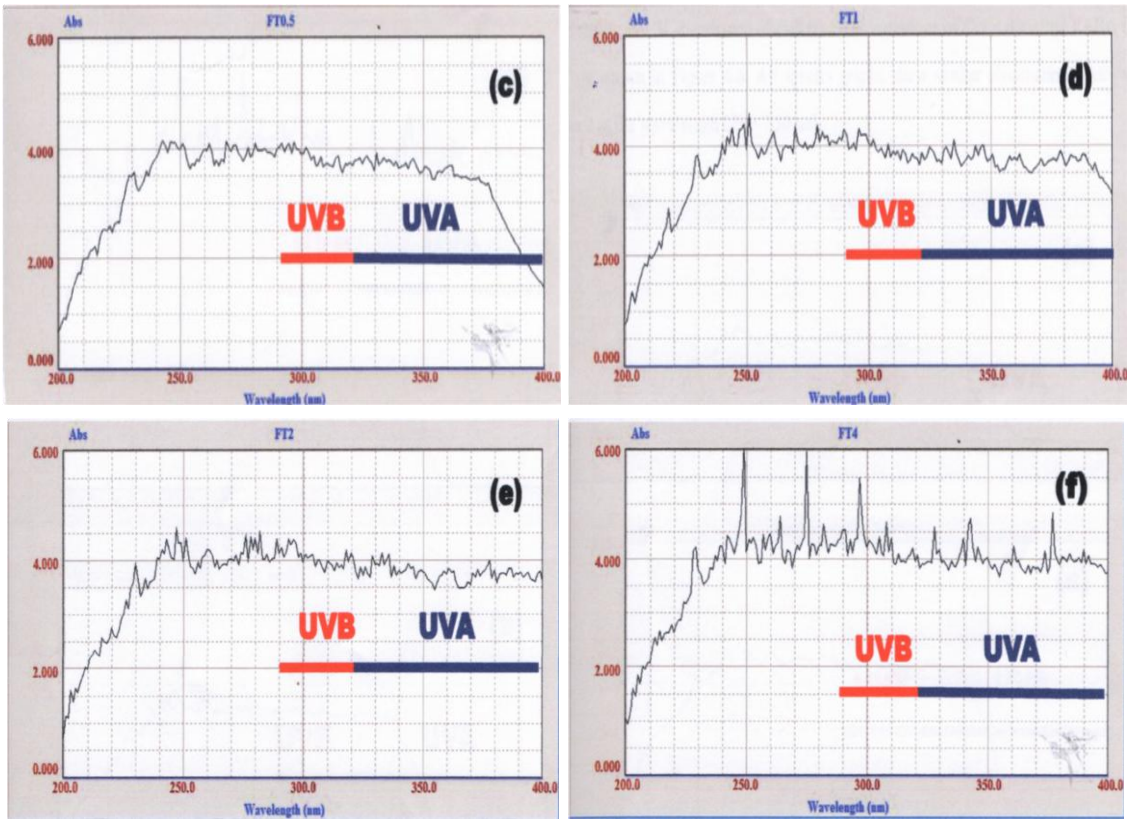

Figure 5. The UV absorbance spectra of *C. chinensis* extract in different concentrations **(c)** 0.50%w/w. **(d)** 1.00 %w/w. **(e)** 2.00 %w/w. **(f)** 4.00 %w/w.

The ability in UV absorbance of different concentrations of Octyl dimethyl PABA (positive control) are shown the ability to sun protection to UVA and UVB. All spectra graphs show similar absorbance values which was show ability in absorption the range of UVA and UVB. The highest concentrations of Octyl dimethyl PABA (7.00 % w/w) was represent in Figure 6.

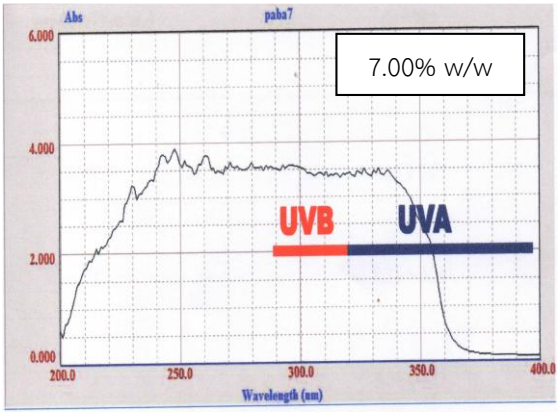

Figure 6. The UV absorbance spectra of Octyl dimethyl PABA at the concentrations 7.00 % w/w.

Discussion

C. chinensis is a parasitic plant which lives with other plants. It has a character of heat tolerance and naturally found in summer or hot climate area.[6] The character of crude extract is gold yellow powder which is similar to the color of fresh plant material. There is a previously study, compared the antioxidant activities between *C. chinensis* extract from the maceration of water and ethanol by assessing their DPPH free radical scavenging, superoxide anion scavenging, anti-superoxide anion formation and anti lipid peroxidation abilities. The flavonol contents of tested samples also were analyzed by high- performance liquid chromatography with an ultraviolet (HPLC-UV) detector. The results showed that there is a direct correlation between flavonol contents and the antioxidant activities from the extracts and fractions of *C. chinensis*. However, *C. chinensis* extract from the maceration with 95% ethanol demonstrated higher amount of extract , significantly better of antioxidant power, and also had a higher flavonol content than *C. chinensis* extract from the maceration with distilled water[3] because the constituents of the *C. chinensis* include flavonol, lignans, quinic acid, and polysaccharide[11] and these constituents belong to the group of compounds with poor water.[12] Therefore, the solvent used to extract *C. chinensis* in this study is ethanol.

First, the antioxidant activity was studied. Antioxidants are secondary metaboliters produced by plant to protect against nutrient-poor environment.[13] The result of Herrmann is reported that there is much higher concentrations of flavones and flavonols in plant which are exposed to sunlight.[14, 15]

The Ferric Reducing Antioxidant Power assay is used to determine the antioxidant power of *C. chinensis* sample which was monitor the change in UV-Vis Spectrophotometer by measuring absorption at 593 nm.

When we calculate FRAP value of tested substance, FRAP value is increased when the concentration of crude extract is increased. So, the antioxidant power of *C. chinensis* is directly relation with some of its compounds in its extracted. Ferric reducing antioxidant power assay may be attributed to the quantities of quercetin and kaempferol compounds in *C. chinensis* extract in each fraction because the main active components of *C. chinensis* in medicated therapy are quercetin and kaempferol[3] and these compounds belong to the group of flavonoid which shows antioxidant activity

Antioxidant is the third type of sunscreen ingredients. Antioxidants can reduce free radicals to less reactive oxygen species forms. During this process the antioxidant itself changes and transforms into a passive radical. A passive radical is relatively stable and can neutralize a second free radical. The antioxidant ability of *C. chinensis* extract posses a tendency to be a good natural sunscreen agent due to its ability in preventing oxidative damage of dermis skin from UV exposure.

If there is an excessive sun exposure, the body may not be able to completely neutralize the free radicals generated by UV exposure, which can lead to photocarcinogenesis and photoaging.

From the determination of Ultraviolet (UV) ray absorption on the UV region by UV-Vis spectrophotometer, the results show that ethanol extract of *C. chinensis* posses a photoprotective efficacy in UV region as showed on spectra graph comparing with negative standard. All concentrations has the clearly similar character of absorbance values at the wavelength 200-240 nm. At wavelength 240-340 nm (UVB), all concentration also has similar spectra but absorbance value was slightly increased it's concentration. At the wavelength 340-400 nm (UVA), the absorbance values showed in spectra was significant increasedin absorption of UVA and UVB wtih the increasing of its concentration.

The spectra character between Octyl dimethyl PABA (Positive control) and *C. chinensis* extract were compared, *C. chinensis* extracts show wider region of UV absorption. Octyl dimethyl PABA shows the absorbance region of UVB while *C. chinensis* extract shows the absorbance of UVA. So, *C chinensis* extract has the ability in UV ray absorption on the UV region higher than Octyl dimethyl PABA at specific region of UVA. At the minimal concentration of *C. chinensis* extract (0.125 % w/w) has the spectra graph similar to Octyl dimethyl PABA at the concentration 1.00-7.00 % w/w since the UV absorption region of Octyl dimethyl PABA at each concentration is quite similar. The *C. chinensis* Lam. extract show the more range in UV ray absorption on the UV region than Octyl dimethyl PABA at all concentration. However, UVC radiation is filtered by the ozone layer, these harmful wavelengths do not reach the earth's surface. So, *C. chinensis* Lam.

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