Photo-stabilization of Sappanwood Ethanolic Extract with Ammonium Alum Chelation

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บทคัดย่อ

สารประกอบฟีนอลิค เป็นที่รู้จักกันดีว่าเป็นสารออกฤทธิ์ที่อยู่ในสารสกัดสมุนไพร โดยปกติแล้ว สารเหล่านี้สามารถสลายได้เมื่อถูกแสง ซึ่งจะทำให้ฤทธิ์ทางชีวภาพของสารนั้นลดลง เพื่อเป็นการเพิ่มความเสถียรต่อแสงให้กับสาร ต้องนำมาทำปฏิกิริยากับสารคีเลตเพื่อป้องการถูกออกซิไดซ์ด้วยแสง งานวิจัยนี้จึงมุ่งเน้นในการใช้แอมโมเนียมอะลัมเพื่อลดการสลายตัวของสารสกัดจากแก่นฝางด้วยแสง ในการศึกษานี้ สารสกัดจากแก่นฝางจะถูกละลายในสารละลายที่ประกอบด้วย แอมโมเนียมอะลัมเอธานอล และฟอสเฟตบัฟเฟอร์ซาลีน พีเอช 7.4 จากนั้นนำไปวางไว้ในห้องที่มีแสงและไม่มีแสงที่อุณหภูมิห้อง เป็นเวลา 48 ชั่วโมง แล้วนำสารละลายดังกล่าวไปวัดค่าการดูดกลืนแสงระหว่าง 200-700 นาโนเมตร จากการศึกษาพบว่า สารละลายที่เติมแอมโมเนียมอะลัม จะมีค่าการดูดกลืนแสงที่ 541 นาโนเมตร ลดลง จาก 3.010 เป็น 2.226 (26.56%) และมีการปรากฏของพีคที่ 573 นาโนเมตร เพิ่มขึ้นมา ในการศึกษาสารละลายที่ไม่ได้ใส่แอมโมเนียมอะลัมและเก็บในที่มืดเป็นเวลา 24 และ 48 ชั่วโมง พบว่า ค่าการดูดกลืนแสงที่ 541 นาโนเมตร ลดลง จาก 3.010 เป็น 0.979 (67.48%) และจาก 3.010 เป็น 0.446 (85.18%) ตามลำดับ ในทางตรงกันข้าม สารละลายที่ไม่ได้ใส่แอมโมเนียมอะลัมและเก็บในที่มีแสงเป็นเวลา 24 และ 48 ชั่วโมง พบว่า ค่าการดูดกลืนแสงที่ 541 นาโนเมตร จะลดลง จาก 3.010 เป็น 0.667 (77.84%) และจาก 3.010 เป็น 0.282 (90.62%) ตามลำดับ ทั้งนี้ เมื่อสารละลายที่มีการใส่แอมโมเนียมอะลัมและเก็บไว้ที่มีแสงเป็นเวลา 24 และ 48 ชั่วโมง พบว่า ค่าการดูดกลืนแสงที่ 541 นาโนเมตร จะลดลงเล็กน้อย จาก 2.226 เป็น 1.908 (14.28%) และจาก 2.226 เป็น 1.200 (46.10%) ตามลำดับ สำหรับการเก็บสารละลายที่มีแอมโมเนียมอะลัมในที่มืดเป็นเวลา 24 ชั่วโมง พบว่า ค่าการดูดกลืนแสงที่ 541 นาโนเมตร เพิ่มขึ้นเล็กน้อย จาก 2.226 เป็น 2.289 (2.82%) ในขณะที่การเก็บไว้เป็นเวลา 48 ชั่วโมง จะมีค่าการดูดกลืนแสงลดลง จาก 2.226 เป็น 1.371 (38.42%) จากการศึกษาสามารถสรุปได้ว่า การเติมแอมโมเนียมอะลัมลงในสารละลายสารสกัดจากแก่นฝาง จะช่วยคงสภาพสารละลายสารสกัดจากแก่นฝางที่เก็บในที่มืด อย่างน้อยเป็นเวลา 24 ชั่วโมง การค้นพบนี้ สามารถช่วยเพิ่มความเสถียรของผลิตภัณฑ์จากสมุนไพรได้ในอนาคต

คำสำคัญ: ความเสถียรต่อแสง สารสกัดจากแก่นฝาง แอมโมเนียมอะลัม การดูดกลืนแสง

Abstract

Phenolic compounds are well-known as active ingredients in herbal extracts. Normally, they are degraded when exposed to light, which decreases their biological activities. To improve their photo-stability, combination with chelating agents is required to prevent photo-oxidation. This study aimed to use ammonium alum to slow the photo-degradation of sappanwood ethanolic extract (SE). Before the

examination, SE was dissolved in a solution of ammonium alum, ethanol, and PBS pH 7.4. After incubation under light and dark conditions at room temperature for 48 h, the solutions were measured to show a photo-absorbance of between 200 to 700 nm. The results revealed that the addition of ammonium alum into SE solutions caused a sudden decrease in the photo-absorbance at 541 nm (PS541) from 3.010 to 2.226 (26.56%) and presented a new peak at 573 nm. Moreover, when unchelated SE solution was maintained under dark conditions for 24 h and 48 h, their PS541 decreased greatly from 3.010 to 0.979 (67.48%) and from 3.010 to 0.446 (85.18%) respectively. When the unchelated SE solution was incubated under light conditions for 24 h and 48 h, their PS541 decreased even more from 3.010 to 0.667 (77.84%) and from 3.010 to 0.282 (90.62%) respectively. However, when the chelated SE solution was incubated under light conditions for 24 h and 48 h, their PS541 was decreased slightly from 2.226 to 1.908 (14.28%) and from 2.226 to 1.200 (46.10%) respectively. For the incubation in dark conditions, the PS54 of the chelated SE solution incubated for 24 h slightly increased from 2.226 to 2.289 (2.82%) while 48 h incubation caused a decrease from 2.226 to 1.371 (38.42%). It can be concluded that ammonium alum chelation stabilized SE for at least 24 h under dark conditions. This finding can be used to improve the photo-stability of herbal products in future studies.

Keywords: Photo-stability; Sappanwood ethanolic extract; Ammonium alum; Photo-absorbance

Introduction

Phenolic compounds are plant secondary metabolites that possess an aromatic ring containing one or more hydroxyl substituents, including esters, methyl ethers, glycosides, and others [1]. Due to them containing numerous aromatic rings, they can absorb electromagnetic radiation (ultraviolet radiation and visible light) and scavenge free radicals. Moreover, their biological activities have been reported, including anti-oxidation, anti-carcinogenicity, anti-inflammation, anti-mutagenicity, anti-microbial proliferation, anti-melanogenesis, and others [1], [2], [3], [4]. Sappanwood (*Caesalpinia sappan* L.) is a perennial plant native to South Asia, Southeast Asia, Africa, and the Americas [5]. Traditionally, it has been used as a medicinal plant in China, India, Myanmar, Vietnam, Sri Lanka, and the Malay Peninsula [2].

In Thai, sappanwood is called “Fang” and has been used as a dye and coloring agent in beverages, food, garments, and the cosmetic industry [6]. Commercially, its extract is contained in Namya Utai, a Thai traditional medicine recipe, to provide red color and pharmacological properties. Moreover, sappanwood extract is used to treat numerous diseases, for example, abscesses, anemia, carbuncles, diarrhea, dysentery, skin infections, tetanus, thrombosis or tumor tuberculosis, and other diseases [7], [8].

Several pharmacological activities of sappanwood extract have been reported, such as anti-bacterial, anti-inflammation, anti-convulsion, anti-oxidation, anti-virus, cell-death protection, hepato-protection, hypoglycemia, and others [2], [5], [6], [8], [9], [10], [11], [12], [13], [14], [15], [16].

The extraction of sappanwood involves various solvents (water, methanol, ethanol, ethyl acetate, and dichloromethane), and brazilin was reported as the main phytochemical of sappanwood [2], [7], [15], [17]. Brazilin is a homo-isoflavonoid corresponding to protosappanin A

and protosappanin B, which are also extracted from sappanwood (Figure 1). However, brazilin can be oxidized by air and light to be brazilein [18]. Furthermore, 8-methoxy bonducellin, caesalpinia chromans, caesalpin J and P, chromanones, chalcones sappanin, ombuin, protosappaninn C, E1 and E2, quercetin, rhamnetin, sappan chalcone, and sappanol were reported as present in sappanwood [19]. Naturally, phenolic compounds rapidly degrade when exposed to light, which decreases their biological activities. To improve their photo-stability, combination with chelating agents is required to prevent photo-oxidation. Alum or aluminum (III) ion can be used to chelate the flavonoid compounds and has been used as the mordant agent in traditional garment staining [18].

This study aimed to use ammonium alum to slow the photo-degradation of sappanwood ethanolic extract (SE).

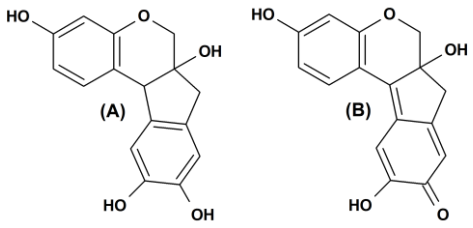

Figure 1 Chemical structure of brazilin (A) and brazilein (B)

Materials and Methods

Extraction of sappanwood heartwood

The extraction of sappanwood heartwood was done by using Soxhlet extractor apparatus. 30 grams of the powdered sappanwood was transferred into a cellulose thimble (Whatman 10350265 Standard 603 Cellulose Extraction Thimble, GE Healthcare, Buckinghamshire, United Kingdom), and then contained in the extraction chamber of a Soxhlet extractor. After that, the lower side of the chamber was connected with a flat-bottomed flask containing 200 mL of 95% ethanol, and the upper side of the chamber was connected with a condenser irrigated by tap water. Subsequently, the flask was placed in boiling water to boil the ethanol. Then, the evaporated ethanol was condensed in the condenser and collected in the chamber until the solvent level in the chamber reached the siphon level. After that, it was drained through the siphon tube into the flask. The process of the powdered sappanwood soaked in the solvent maintained in the chamber and then drained into the flask was counted as 1 cycle. In the experiment, the extraction was carried out in 20 cycles for each batch. Six batches of the extraction were combined together before evaporating the solvent by use of a rotary evaporator (Rotavapor R-210/R-215, BÜCHI Labortechnik AG, Flawil, Switzerland). To eliminate the solvent, the evaporating flask that contained the crude extract was partially immersed in a 45ºC water bath and rotated at 70 rpm. Next, the pressure was slowly reduced until it reached 100 mbar to facilitate the evaporation of ethanol. The evaporation was performed until the concentrated extract and the viscose extract were collected. Then, the extract was transferred into an evaporation disk and covered by punctured aluminum foil. Finally, the disk was placed in a hot air oven at 45ºC until a constant weight was obtained. The crude extract was collected and protected from light and air exposure until further use.

Preparation of chelated SE

The sappanwood ethanolic extract (SE) was dissolved in 0.1 mg/mL aqueous solution that

contained 10% of ethanol, 10 mM of phosphate buffered saline pH 7.4 (PBS pH 7.4), and 0.04 mg/mL of ammonium aluminum sulfate dodecahydrate (ammonium alum or $AlNH_4(SO_4)_2 \cdot 12H_2O$), which provided a molar ratio between brazilein (assumed as SE composed by 100% of brazilein) and ammonium alum as 1:1. Before the preparation of chelated SE, 0.5 mg/mL SE in 50% ethanol, 0.4 mg/mL ammonium alum, and 100 mM PBS pH 7.4 were prepared. After that, they were mixed together in a micro-centrifuge tube with distilled water (600 µL), 0.5 mg/mL SE in 50% ethanol (200 µL), 0.4 mg/mL ammonium alum (100 µL), and 100 mM PBS pH 7.4 (100 µL) to obtain 1,000 µL of mixture volume. The extract was chelated with the aluminum (III) ions to be chelate compounds such as Al $(brazilein)_2$ (Figure 2) [18].

Characterization of chelated SE

After the chelated SE was prepared, it was simultaneously incubated under light conditions (approximate 250 lux from a fluorescent lamp) and under dark conditions at room temperature. Oxygen was limited by closing the micro-centrifuge tube. The tubes were maintained for 0, 24, and 48 h. before measuring the photo-absorbance every 1.0 nm between 200 to 700 nm with 750 nm/min scan speed by use of a spectrophotometer (Biochrom Libra S21 Visible Spectrophotometer, Biochrom Ltd., Cambridge, United Kingdom). Finally, the photo-absorbances at 541 nm (PS541) of the mixtures were averaged and the standard deviations were calculated. The study was done in triplicate.

Table 1 Photo-absorbance at 541 nm (PS541) of the unchelated SE and the chelated SE

Alum addition	Incubation time (h)	Incubation condition	PS541 (OD. 541 nm)	Standard deviation
Without alum	0	-	3.010	0.025
	24	Light	0.667	0.055
		Dark	0.979	0.017
	48	Light	0.282	0.029
		Dark	0.446	0.004
With alum	0	-	2.226	0.071
	24	Light	1.908	0.052
		Dark	2.289	0.014
	48	Light	1.200	0.103
		Dark	1.371	0.027

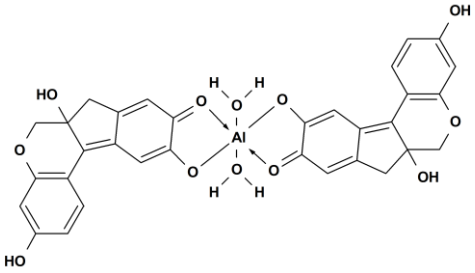

Figure 2 Structure of Al(brazilein)$_2$ complex from brazilein and aluminum (III) ion complexation [18]

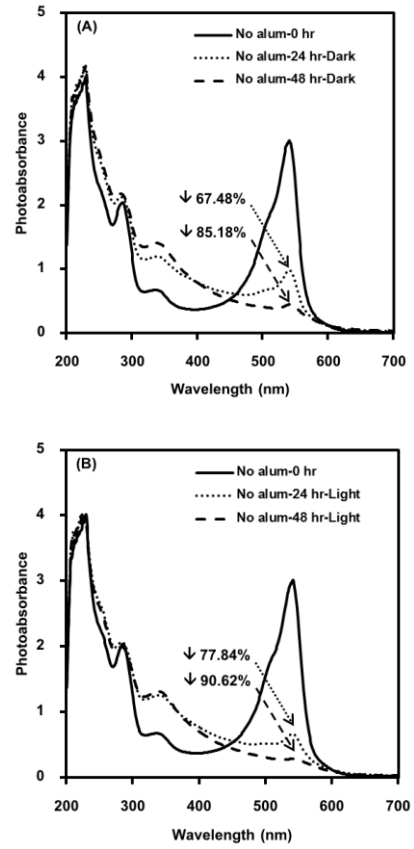

Figure 3 Photo-absorbance of the unchelated SE incubated under dark conditions (A) and light conditions (B)

Results and Discussion

Extraction of sappanwood heartwood

Six batches of the crude extracts were collected from the extraction of sappanwood heartwood in 95% ethanol by the use of Soxhlet extraction apparatus, and 30.24 g of dried crude sappanwood ethanolic extract was obtained.

Characterization of chelated SE

The average data and its standard deviations from the measurements of photo-absorbances at 541 nm (PS541) of both chelated and unchelated sappanwood ethanolic extracts (SE) are shown in Table 1. The results revealed that although the unchelated SE solution was maintained under dark conditions, PS541 of the solution decreased greatly from 3.010 to 0.979 (67.48%) for 24 h incubation, and decreased more from 3.010 to 0.446 (85.18%) for 48 h incubation (Figure 3.A). When the unchelated SE solution was kept under light conditions for 24 h and 48 h, their PS541 decreased from 3.010 to 0.667 (77.84%) and from 3.010 to 0.282 (90.62%) respectively (Figure 3.B).

The results showed the addition of ammonium alum into the SE solution influenced PS541 of the SE solution, indicating a decrease from 3.010 to 2.226 (26.56%) (Table 1). However, there was a new peak at 573 nm after the chelation and this nearly disappeared after 48 h incubation under both dark and light conditions (Figure 4). Nevertheless, when the chelated SE solution was incubated under dark conditions for 24 h, PS541 slightly increased from 2.226 to 2.289 (2.82%) (Figure 4A). Meanwhile, 48 h incubation produced a decrease from 2.226 to 1.371 (38.42%) (Figure 4A). For the incubation of the chelated SE solutions under light conditions for 24 h and 48 h, PS541 decreased from 2.226

to 1.908 (14.28%), and from 2.226 to 1.200 (46.10%) respectively (Figure 4B).

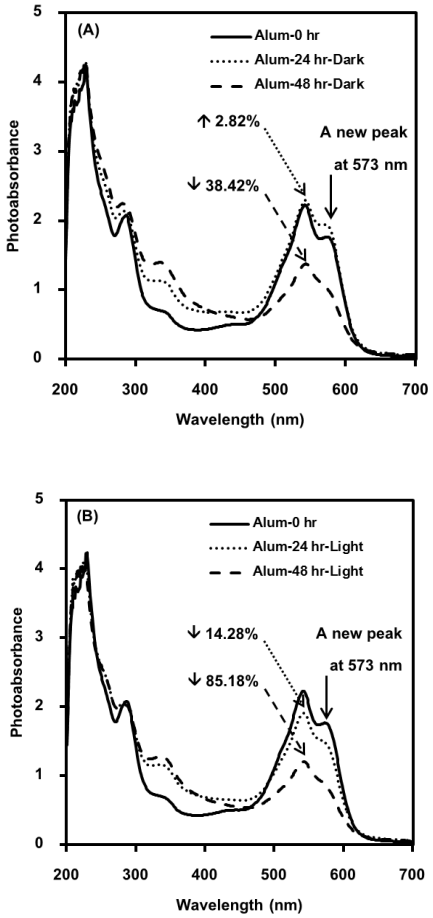

Figure 4 Photo-absorbance of the chelated SE incubated under dark conditions (A) and light conditions (B)

Conclusion

Although the unchelated SE was incubated under dark conditions at room temperature, their photo-absorbance decreased for 77.84% after 24 h incubation. However, the chelation of SE by using ammonium alum stabilized SE solution for at least 24 h under dark conditions. This study showed that the stability of products comprising SE could be improved in future studies. Furthermore, this finding could be applied to maintain the efficacy of other herbal products.

Acknowledgements

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