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UV protection effect of cotton dyed with Flos Sophorae (*Sophora Japonica* L.) extracted with acid hydrolysis

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Abstract

Flos Sophorae extract was hydrolyzed using a mixed solution of methanol, HCl, and water to convert rutin to quercetin for the purpose of enhancing the UV protective effect of dyed cotton. Relative yield (%) of quercetin in the hydrolyzed extract was determined by the percent ratio of the absorbance of untreated Flos Sophorae extract at 359 nm to the absorbance of hydrolyzed extract at 370–371 nm. Quercetin yield was 93.09% when 1 M HCl was applied and 52.03% when 0.5 M HCl was applied, both with 60 min reaction time. The latter was applied in dyeing cotton with the dyebath containing hydrolyzed Flos Sophorae extract and water by 50:50 or 75:25 ratio. By dyeing with hydrolyzed Flos Sophorae extract the UPF value of cotton was enhanced to 13.80 indicating 92.76% blockage of the UV rays as opposed to 84.78% blockage by the same fabric without Flos Sophorae dyeing. The present method could produce a lightweight summer cotton with yellow color which could provide a moderate UV protection for daily summer attire.

Keywords: Flos Sophorae, Dyeing, Cotton, Acid hydrolysis, UV Protection, UPF, Quercetin, Rutin

Introduction

Solar ultraviolet (UV) radiation is an essential element for human body. Nevertheless, overexposure can cause skin problems and diseases such as sunburn, erythema, and skin cancer (Xin et al., 2004). In everyday life, UV protection is achieved by clothing or other textile items covering the body in addition to the use of sunscreen lotions (Saravanan, 2007). Throughout the year UV radiation peaks during April to August and this is when people wear lighter clothing and involve in more outdoor activities (United States Environmental Protection Agency, 2004).

Generally, clothing made of thick fabric with dense structure and dark color can provide a fair amount of UV protection (Saravanan, 2007). However, lightweight, light-colored, and porous fabric which is often used during summer cannot provide the necessary UV protection by itself (Dubrovski, 2010; Saravanan, 2007). Cotton which is the most popular textile material all year around has poor UV protection and even lower protection when it is bleached (Saravanan, 2007). UV protection of cotton can be enhanced if the natural pigment of cotton is still present, fluorescent whitening agent is added, and inorganic or organic UV absorbers are added to cotton (Gorjanc et al., 2014).

The effect of UV protection of fabric is represented by the Ultraviolet Protection Factor (UPF) (Dubrovski, 2010; Kim, 2016; Sungrubbies, 2022). UPF value is primarily used for designating the UV protection of garments which are specifically designed for this purpose and produced from a fabric that is rated of its UV protection ("Sun protective clothing," 2021). From the UPF value, it is possible to understand how much a fabric can block UV from the sun. The UPF concept can be easily understood by the EWT (Erythema weighted transmittance) value which explains the degree of penetration of UV through the fabric (Dubrovski, 2010). EWT has the value from 0 to 1 or 0% to 100% and it is designated by the inverse value of UPF (Eq. 1) (Dubrovski, 2010).

$$EWT = \frac{1}{UPF} \tag{1}$$

The UPF rating system along with the allowed textile labels designated by the Australian/New Zealand standard, ASTM D6544, ASTM D6603, AATCC 183 etc. is shown in Table 1 (Gorjanc et al., 2014; Narantuya & Ahn, 2020; Sungrubbies, 2022; "Sun protective clothing," 2021). UPF value of 15 or above is required for a garment to receive the label designating the UV protective garment (Crews & Hustvedt, 2005). The mean UPF value of bleached cotton was 4.12 or lower while that of cotton dyed into black, navy or red was 18–37 (Crews & Hustvedt, 2005; Gorjanc et al., 2014; Saravanan, 2007). Dyeing cotton with natural dye extracted from plant increased the UPF value especially when alum mordant was used in comparison to using $CuSO_4$ or $FeSO_4$ (Gupta et al., 2005; Saravanan, 2007).

Sophora Japonica L. (also known as pagoda tree) is widely distributed in Asia, Europe, North America (Gong et al., 2021). Its dried flower bud Flos Sophorae has been used traditionally for dyeing textiles yellow in the presence of aluminum mordant (Chu & Soh, 2002; Gong et al., 2021; Narantuya & Ahn, 2020). The main coloring compounds of Flos Sophorae are quercetin ($C_{15}H_{10}O_7$) and its glycoside rutin ($C_{27}H_{30}O_{16}$) which are the phenolic compounds responsible for the antioxidant, anti-inflammatory, and UV protective characteristics of Flos Sophorae (Fig. 1) (Gong et al., 2021; Narantuya, 2020; Paniwnyk et al., 2001; Vetrova et al., 2017; Zhou & Tang, 2017). Quercetin and rutin are widespread in various fruits and vegetables such as onion, tomato, grape, green tea, etc. (Cefali et al., 2016; Lakhanpal & Rai, 2007).

Table 1 UPF ratings and corresponding labels allowed for UV protective clothing

UPF Rating	Protection Category	% UV radiation blocked	UPF values allowed on the labels
UPF 0 ~ 14	Non-rateable	Less than 93.3	n.a
UPF 15 ~ 24	Good	93.3–95.9	15 and 20
UPF 25 ~ 39	Very Good	96.0–97.4	25, 30, and 35
UPF 40 ~ 50 +	Excellent	97.5–98.0 +	40, 45, 50, and 50 +

(Gorjanc et al., 2014; Narantuya & Ahn, 2020; Sungrubbies, 2022; "Sun protective clothing," 2021)

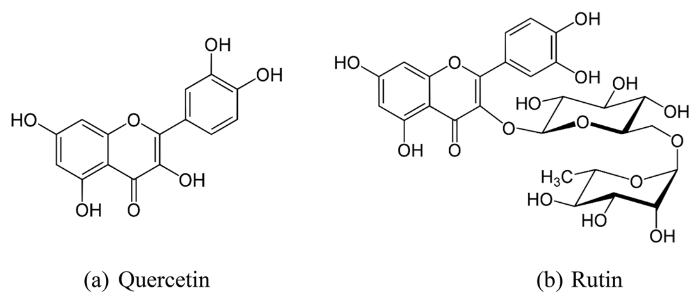


Fig. 1 Structure of Quercetin ("Quercetin," 2022) and rutin ("Rutin," 2021)

Among the many UV protective compounds found in plants, quercetin and rutin are the most extensively studied substances (Cefali et al., 2016). Quercetin has higher UV protection over rutin in both SPF (Sun protection factor) and UPF (Ultraviolet protection factor) values (Choquenet et al., 2008; Kostyuk et al., 2018; Monsalve-Bustamante et al., 2020; Narantuya & Ahn, 2020; Zhou & Tang, 2017). Currently, quercetin is a widely used sunscreen agent in many facial cosmetic products (INCIDecoder, 2022)

Owing to the physiological effect of quercetin and rutin, dyeing cotton with Flos Sophorae would produce a yellow colored cotton with antioxidant, anti-inflammatory, and UV protective function. The physiological effect would be enhanced if the plant contained high amount of quercetin. However, the composition of rutin in Flos Sophorae is higher than that of quercetin since phenolic compounds are present in plant in the form of glycoside (Vetrova et al., 2017). It was found that the proportion of rutin to quercetin in Flos Sophorae was 13:1 or as high as 50:1 depending on the extraction method (Vetrova et al., 2017). The fact that Flos Sophorae contains higher amount of rutin than quercetin presents limitation on applying Flos Sophorae as an effective UV protective natural dye on textiles.

Research has been carried out in the pharmaceutical and biotechnical area for the conversion of rutin in plants into quercetin or isoquercitrin using methods such as subcritical water extraction and acid or enzyme hydrolysis (Nam et al., 2012; Turner et al., 2006; Wang et al., 2011; Yang et al., 2019). When hydrolysis method was used rutin was readily converted to quercetin by the cleavage of disaccharide and with lower yield it was converted to isoquercitrin by the cleavage of deoxy sugar (Fig. 2) (Isaak et al., 2013; Wang et al., 2011).

The highest yield of quercetin was observed with acid hydrolysis using hydrochloric acid (HCl) as the catalyst (Wang et al., 2011). By applying this method in textile dyeing rutin in Flos Sophorae extract can be hydrolyzed to quercetin, allowing higher composition of quercetin in the dyebath of Flos Sophorae extract. Cotton dyed by the hydrolysis of Flos Sophorae would produce cotton fabric that has yellow color and enhanced UV protective effect as well as antioxidant and anti-inflammatory functions. This research was aimed to investigate the method of acid hydrolysis of rutin in Flos Sophorae extract for the purpose of producing light-yellow-colored summer cotton with enhanced UV protection.

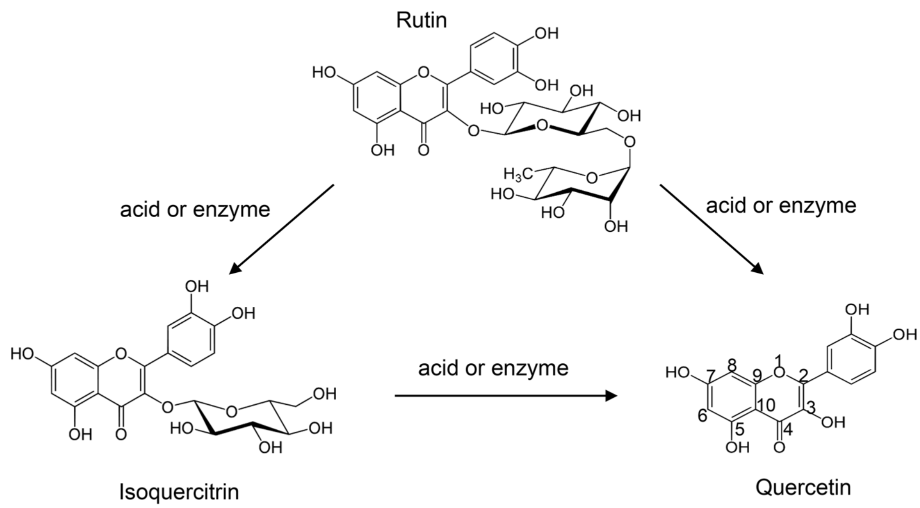


Fig. 2 Conversion of rutin to quercetin or isoquercitrin. Aadapted from Nam et. al. (2012), Isaak et. al. (2013), and Baby et. al. (2016)

Materials and methods

A plain weave cotton fabric (thickness 0.15 mm, weight 70 g/m^2, fabric count 98 × 90/inch^2) was purchased from Sombe (Korea). Flos Sophorae and potassium alum ($KAl(SO_4)_2 \cdot 12H_2O$) mordant were purchased from Arts and Crafts (Korea). Quercetin (CAS No 117-39-5) and Rutin (CAS No 207671-50-9) standards were purchased from Sigma Aldrich (USA). Methanol (HPLC grade) was from J. T. Baker (USA) and HCl was from Daejung Chemicals & Metals (Korea). Glass microfiber filter (90 mm diameter) was from Whatman (UK), nylon syringe filter (0.45 µm) was from Grace (USA). Water used throughout the experiment was distilled by Human Corporation (Korea).

Experimental methods

Examination of rutin and quercetin yield from Flos Sophorae extract

A 2 g of Flos Sophorae powder was extracted with 200 mL of water at 40 °C, 75 °C, and 90 °C for 1 h using Soxhlet waterbath (LK Labkorea, Korea). 20 mL of this extract was taken and water was evaporated using the rotary evaporator (Eyela N-1300, Germany) at 45 °C and 50 rpm. The precipitate was dissolved in 20 mL of methanol and filtered using the 0.45 µm nylon syringe filter for the analysis of high performance liquid chromatography diode array detector (HPLC–DAD).

Dilutions of Rutin standard and Quercetin standard purchased from Sigma Aldrich (USA) were prepared by dissolving 2 mg/L, 20 mg/L, 100 mg/L, and 200 mg/L each. Standard dilutions were analyzed using the HPLC–DAD instrument to confirm the retention time (Rt) and the λ_{max} of rutin and quercetin compounds. Using the area (mAU min) of the peaks the standard graphs of rutin compound and quercetin compound were prepared. The regression equation deduced from the standard graphs were used to calculate the concentration of rutin and quercetin compounds.

Hydrolysis of rutin compound in Flos Sophorae

A 30 mL of methanol was mixed with 20 mL of either 0.5 M or 1 M of HCl in water. 2 g of Rutin standard was added to each of the mixed solution. The mixture was reacted in the Soxhlet waterbath for 30 or 60 min at 90 °C, then filtered using a glass microfiber filter. The liquid was evaporated using the rotary evaporator at 75 °C and 50 rpm. The precipitate was dissolved with 50 mL of methanol and filtered using a 0.45 μm nylon syringe filter.

Flos Sophorae (0.5 g) was added to the mixed solution of methanol (30 mL) and either 0.5 M or 1 M of HCl (20 mL). The mixture was reacted in the Soxhlet waterbath for 30 or 60 min at 90 °C, then filtered using the glass microfiber filter. The liquid was evaporated using the rotary evaporator at 75 °C and 50 rpm. The precipitate was dissolved in 50 mL of water. Water was evaporated in the rotary evaporator, then the precipitate was dissolved in methanol and filtered using the 0.45 μm nylon syringe filter.

A 0.02 g each of Rutin and Quercetin standards were dissolved in 100 mL methanol to prepare Rutin and Quercetin standard solutions. The absorbance (λ_{max}) of Rutin and Quercetin standard, hydrolyzed Rutin standard solution, hydrolyzed Flos Sophorae extract were examined using the UV–Vis spectrophotometer (Lambda-25, Perkin Elmer, USA).

Cotton dyeing by different extractions of Flos Sophorae

Flos Sophorae (2 g) in 200 mL water was extracted using the Soxhlet waterbath for 1 h at 90 °C. The extract was filtered using the glass microfiber filter and was used as the untreated Flos Sophorae extract.

Flos Sophorae (2 g) was added to the mixed solution of 120 mL of methanol and 80 mL of 0.5 M HCl in water. The extraction and hydrolysis were carried out in the Soxhlet waterbath at 90 °C for 1 h. The reactant was filtered using the glass microfiber filter, then the liquid was evaporated by the rotary evaporator. The precipitate was taken with 200 mL of water and used as the hydrolyzed Flos Sophorae extract.

Flos Sophorae (2 g) in 200 mL of methanol was treated in Soxhlet waterbath at 90 °C for 1 h. The extract was filtered using glass microfiber filter. Liquid was evaporated using the rotary evaporator and the precipitate was taken with 200 mL of 50 °C water.

Dyebath of Flos Sophorae extract was prepared by mixing three different extractions prepared above with water by 50:50 and 75:25 ratio. Cotton (10 cm × 10 cm, 0.67 g) was dyed using the above dyebath using the simultaneous mordanting method. A 1 g/L of alum mordant was added to the dyebath and dyeing was conducted for 30 or 60 min at 70 °C. For each dyeing condition, three samples dyed in individual dyebath. Dyed sample was washed in tap water and air dried.

Analysis of experimental results

Color measurement

CIELAB Color values (L^*, a^*, b^*, ΔE^*) of dyed cotton was measured using the spectrocolorimeter (Color i5, X-rite, USA) under D_{65} illuminant and 10° standard observer at three spots of each sample. The K/S value obtained from the maximum absorbance wavelength (λ_{max}) was used as the color strength value.

UPF measurement

The UV protection effect of dyed samples was measured using the Ultraviolet transmittance analyzer (UV-2000F, Labshpere Co., USA) at 3 spots of each sample. The measurements were taken according to the AATCC 183:2000 standard. For each measurement, UVA transmittance at 320–400 nm and UVB transmittance at 280–320 nm were obtained. The UPF value was calculated by the software of UV-2000F instrument (Dubrovski, 2010).

Test of colorfastness

The test of colorfastness to sunlight (KS K ISO 105-B01) and the test of colorfastness to laundering (KS K ISO 105-C01) were carried out on the dyed cotton samples using the Launder-O-Meter (Samwoo Kurex, Korea) and the Xenon Weather-O-Meter (S3000, Atlas, USA).

HPLC–DAD analysis

HPLC analysis was conducted using the Ultimate 3000 (Dionex, USA) HPLC equipped with the diode array detector. Separation of compounds were achieved by VDSpher C-18 column, flow rate was 0.8 mL/min, and oven temperature was 30 °C. The gradient mode applied using buffer A (0.3% Trifluoroacetic acid) and buffer B (acetonitrile) was: from 0 to 25 min A:B was 90:10, from 25 to 30 min A:B was 35:65, from 30 to 35 min A:B was 0:100, from 36 to 40 min A:B was 90:10. Total running time was 40 min and the DAD scanning was at 190 to 400 nm.

Statistical analysis

Student t-test was conducted to compare the means of two independent group of samples using the SPSS statistics software (v. 25).

Results and discussion

Determination of extraction temperature for high yield of rutin and quercetin compounds

Rutin standard and Quercetin standard dissolved in methanol were examined using the HPLC–DAD analysis (Table 2). Rutin standard showed a prominent peak at 17.8 min retention time (*Rt*) in the HPLC chromatogram and the UV spectrum of the peak obtained from the DAD analysis showed maximum absorbance (λ_{max}) at 256.5 nm and 355.3 nm. Quercetin standard showed the major peak at 29.6 min and the UV spectrum showed maximum absorbance at 256.5 nm and 370.5 nm. These *Rt* and λ_{max} were used to identify rutin and quercetin compounds in Flos Sophorae extract.

Standards dilutions (2 mg, 20 mg, 100 mg, 200 mg/L) of Rutin and Quercetin standards were examined using the HPLC–DAD instrument. Using the area (mAU min) of the peaks of rutin and quercetin compounds in each standard dilution the standard graphs were generated. From the graphs the regression equations $y = 0.3197x - 0.0118$ ($R^2 = 1$) and $y = 0.6299x + 0.2098$ ($R^2 = 1$) were obtained for rutin and quercetin compounds respectively (Fig. 3). The equations were used to calculate the concentration of rutin and quercetin compounds in Flos Sophorae extract.

Flos Sophorae extracted in water for 60 min using 3 different temperature (40, 75, 90 °C) was examined by the HPLC–DAD. The result of 90 °C extract is shown in Fig. 4.

Table 2 HPLC–DAD results of Rutin standard and Quercetin standard

	Peak compound	Rt (min)	λ_{max} (nm)
Rutin standard	Rutin	17.8	256.5 355.3
Quercetin standard	Quercetin	29.6	256.5 370.5

Two major peaks appeared in the HPLC chromatograms at 17.8 min and 29.6 min. The retention times of the peaks matched the peaks of rutin and quercetin compounds observed in the chromatograms of Rutin and Quercetin standards.

The concentration of rutin and quercetin compounds in each extract was quantified by inputting the corresponding peak area to the regression equations of rutin and quercetin compounds identified the standard solutions (Table 3). The concentration of rutin (123.87 mg/L) and quercetin (16.16 mg/L) compounds was the highest when the extraction temperature was 90 °C. It was over 2.5 times higher than the rutin compound in the 40 °C extract (49.13 mg/L) and over 1.9 times higher than the rutin compound in 75 °C extract (65.91 mg/L) of Flos Sophorae. The result indicated that the concentration of rutin was 4.60–7.66 times higher than that of quercetin. This result was comparable to Liu et. al. (2016) who found that the amount of rutin was about 4.7–9.8 times higher than that of quercetin when Flos Sophorae was extracted by different concentrations of

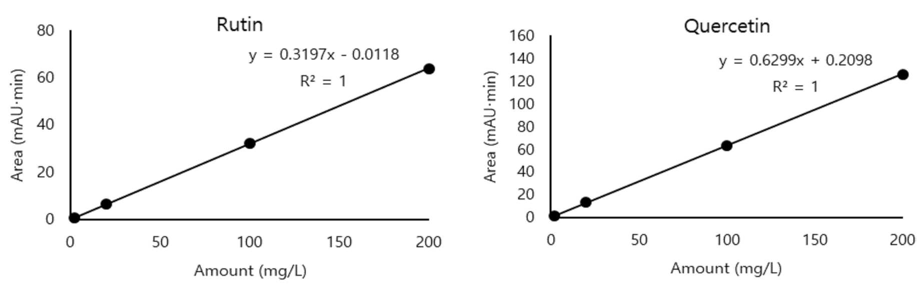


Fig.3 Standard graphs of rutin and quercetin obtained from the HPLC–DAD analysis

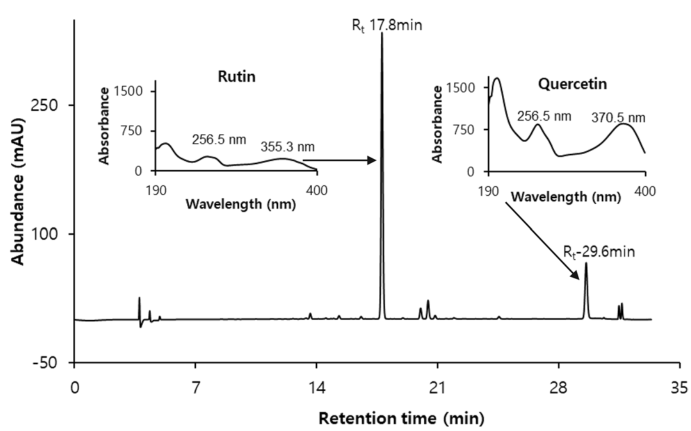


Fig. 4 HPLC chromatogram and the UV spectra of Flos Sophorae extracted by water at 90 °C

methanol and ethanol. Based on the results, the extraction temperature of 90 °C was chosen for the following experiment.

Determination of hydrolysis condition for high yield of quercetin compound

Hydrolysis of rutin standard

Rutin standard was dissolved in the mixed solution of methanol, HCl (0.5 or 1 M), and water and left to hydrolyze at 90 °C for 30 or 60 min. The absorbance spectrum of the reactant was examined using the UV–Vis spectrophotometer. The conversion of rutin compound to quercetin compound in Rutin standard was verified by comparing the λ_{max} of the hydrolyzed Rutin standard to the λ_{max} of untreated Rutin (359 nm) and Quercetin standards (371 nm). UV absorbance spectra of Rutin standard hydrolyzed by 4 different combinations of HCl concentration and reaction time indicated that rutin compound in the reactant was successfully converted to quercetin compound. This was represented by the shift of λ_{max} from 359 nm to 370–371 nm (Figs. 5, 6).

The λ_{max} of quercetin compound was in good agreement with the λ_{max} reported by Stanojević et. al. (2017). Isoquercitrin which is another compound that can be produced from the hydrolysis of rutin was reported to have the maximum UV absorption at 354 nm by Freitas et. al. (2016) and Kim et. al. (2017). In the present investigation the shift of λ_{max} of rutin was confined to 370–371 nm which indicated that isoquercitrin was not produced. Quercetin was the sole product converted from the hydrolysis of rutin.

According to the Beer-Lambert Law the absorbance of a solution is directly proportional to the concentration of the solute and the length of light path (Bhanvase & Barai, 2021). The higher the absorbance, the higher the concentration of the solute (Bhanvase & Barai, 2021). Following the Beer-Lambert Law it is possible to deduce the relative concentration of two different solution using their UV absorbance values (Bhanvase & Barai, 2021). Based on the premise of the Beer-Lambert Law (Bhanvase & Barai, 2021), the relative yield (%) of quercetin compound was determined by the percent ratio of the absorbance of untreated Rutin standard at 359 nm to the absorbance of hydrolyzed Rutin standard at 370 ~ 371 nm (Table 4).

The relative yield of quercetin compound was 95.89% when HCl concentration was 1 M and the reaction time was 60 min. The relative yield was 94.52% when HCl concentration was 0.5 M and the reaction time was 60 min, slightly lower than that obtained by the hydrolysis reaction using 1 M HCl. Yang et. al. (2019) said that rutin was completely

Table 3 Concentration of rutin and quercetin in Flos Sophorae extract obtained by different extraction temperatures

Extraction temperature	Compound	Retention time (min)	Area (mAU min)	Concentration (mg/L)	Ratio rutin/quercetin
40 ℃	Rutin	17.8	15.695	49.13	4.60
	Quercetin	29.6	6.927	10.66	
75 ℃	Rutin	17.8	21.061	65.91	4.81
	Quercetin	29.6	8.824	13.68	
90 ℃	Rutin	17.6	39.589	123.87	7.66
	Quercetin	29.6	10.386	16.16	

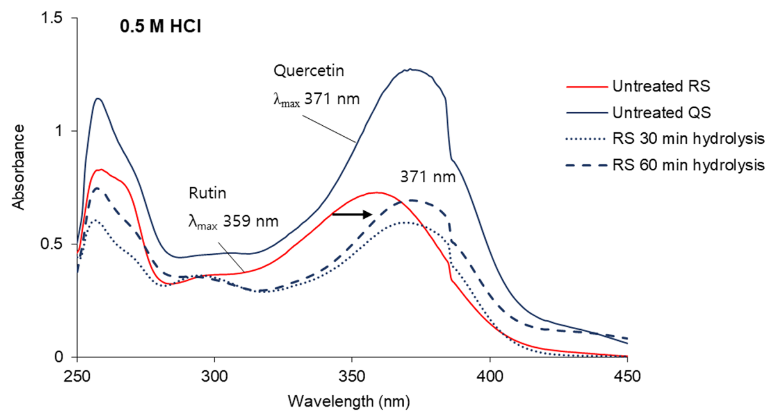


Fig. 5 UV spectra of Rutin standard (RS) hydrolyzed with 0.5 M HCl and Quercetin standard (QS)

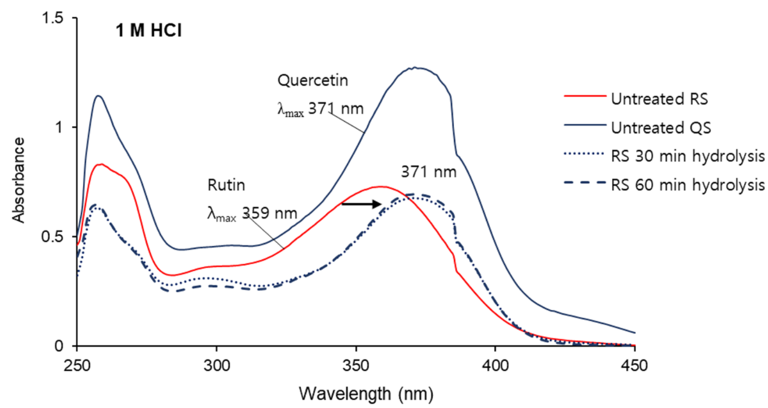


Fig. 6 UV spectra of Rutin standard (RS) hydrolyzed with 1 M HCl and Quercetin standard (QS)

converted to quercetin after 3 h of hydrolysis reaction with 80% ethanol and 0.5 M HCl and that the speed of hydrolysis reaction became faster with the increase in HCl concentration. The present result was comparable to that of Yang et. al. (2019) considering that the reaction time applied in this research was much shorter. The concentration of HCl and the reaction time utilized in this research successfully converted rutin to quercetin and 1 M over 0.5 M HCl concentration and 60 min over 30 min reaction time gave higher yield of quercetin.

Hydrolysis of Flos Sophorae

Flos Sophorae was treated with the mixed solution of methanol, HCl (0.5 or 1 M), and water at 90 °C for 30 or 60 min and the absorbance spectrum of the reactant was examined using the UV–Vis spectrophotometer (Figs. 7, 8). The λ_{max} of hydrolyzed Flos Sophorae was compared with the λ_{max} of Flos Sophorae extracted by water without the hydrolysis treatment. Comparison was also made with the λ_{max} of Flos Sophorae extracted by methanol without the hydrolysis treatment. The latter comparison was made to ensure that methanol in the reactant did not have any side effect on the λ_{max} of Flos Sophorae extract. The λ_{max} of untreated Flos Sophorae extracted by water was

Table 4 Result of hydrolysis of Rutin standard

Sample		λ_{max} (nm)	Absorbance	Relative yield of quercetin (%)
Untreated Rutin standard		359	0.73	–
Untreated Quercetin standard		371	–	–
Hydrolyzed Rutin standard				
0.5 M HCl	30 min	370	0.59	80.82
	60 min	371	0.69	94.52
1 M HCl	30 min	371	0.68	93.15
	60 min	371	0.70	95.89

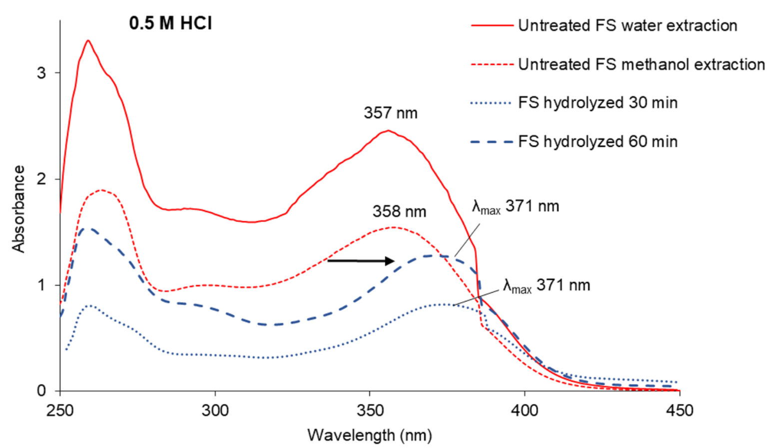


Fig. 7 UV spectra of Flos Sophorae (FS) hydrolyzed with 0.5 M HCl and Untreated Flos Sophorae extracted by water and methanol

357 nm and Flos Sophorae extracted by methanol was 358 nm both of which showed a 1–2 nm difference compared to the Rutin standard (359 nm). Nonetheless it was obvious that the observed λ_{max} of untreated Flos Sophorae water extract and methanol extract represented the UV absorbance of rutin compound. A slight shift of λ_{max} might be due to the impurities within the Flos Sophorae.

The absorbance of the extract was higher when Flos Sophorae was extracted by water (2.46) than when it was extracted by methanol (1.54) (Table 5). This result supports the water extraction of Flos Sophorae for hydrolysis reaction and further dyeing. The highest yield of quercetin (93.09%) was obtained when the water extraction of Flos Sophorae was hydrolyzed with 1 M of HCl for 60 min. Relative yield of quercetin in the extract was much lower when the HCl concentration used in the hydrolysis reaction was 0.5 M (52.03%). This result was in agreement with Wang et. al. (2011) who reported that the HCl hydrolylsis of rutin gave dramatically high yield of quercetin when the acid concentration used was 1 M compared to 0.5 M. Based on the literatures, it is predicted that the hydrolysis reaction of Flos Sophorae would be faster (Yang et al., 2019) and the yield of quercetin compound would be higher (Wang et al., 2011) if 1 M HCl is used in the hydrolysis reaction. In this research however 0.5 M of HCl was selected for the hydrolysis of Flos Sophorae for dyeing to see whether the UPF of cotton was still enhanced

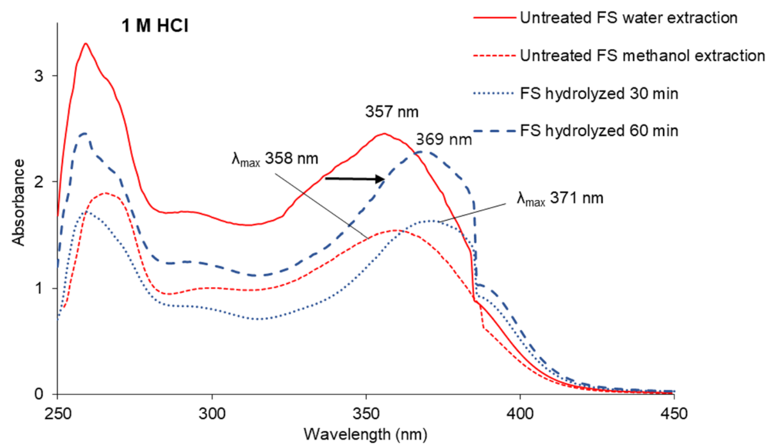


Fig. 8 UV spectra of Flos Sophorae (FS) hydrolyzed with 1 M HCl and Untreated Flos Sophorae extracted by water and methanol

Table 5 Result of hydrolysis of Flos Sophorae extracted by water

Sample	Reaction time	λ_{max} (nm)	Absorbance	Relative yield of quercetin (%)
Untreated Flos Sophorae water extract		357	2.46	–
Untreated Flos Sophorae methanol extract		358	1.54	–
Hydrolyzed Flos Sophorae				
0.5 M HCl	30 min	371	0.82	33.33
	60 min	371	1.28	52.03
1 M HCl	30 min	371	1.63	66.26
	60 min	369	2.29	93.09

with a milder hydrolysis condition which would have lesser effect on cotton and the environment.

Color and UV protection of cotton dyed with hydrolyzed Flos Sophorae extract

Color of dyed cotton

Flos Sophorae extract hydrolyzed with 0.5 M HCl for 60 min was used as the stock solution for dyeing and it was labeled FS. FS was mixed with water (DW) by 50:50 or 75:25 ratio. Dyeing was carried out at 70 °C for 30 or 60 min based on the preliminary investigation which indicated that 70 °C dyeing gave stronger yellowness (b^*) and higher color strength (K/S) than 90 °C when cotton was dyed with Flos Sophorae extract, Rutin standard, and Quercetin standard (Narantuya, 2020). Also, simultaneous mordanting was applied using 1.0 g/L of potassium alum based on the preliminary investigation which indicated that better UPF value was obtained by the above condition compared to higher concentration and pre- or post-mordanting (Narantuya, 2020). The result was compared with the color values of cotton dyed with untreated Flos Sophorae extract and also with the color values of cotton dyed with Rutin and Quercetin using the same dyeing method.

Color of cotton dyed with hydrolzed Flos Sophorae extract resulted in higher color difference (ΔE^*) and color strength (K/S) in all combinations of dyebath type and dyeing time compared to the untreated Flos Sophorae extract (Table 6). Mean ΔE^* of cotton dyed with hydrolyzed Flos Sophorae extract was 81.63 which was 1.10 times higher than that of cotton dyed with untreated Flos Sophorae extract. Mean K/S value of cotton dyed with hydrolzed extract was 9.79 which was 1.58 times higher than that of cotton dyed with untreated extract. The highest K/S value was observed in cotton dyed with the hydrolyzed FS:DW 50:50 dyebath for 60 min (11.11) followed by the FS:DW 75:25 dyebath for 60 min (9.72).

Mean K/S value of the cotton dyed with hydrolyzed extract (9.79) was comparable to and even slightly higher than that of the cotton dyed with Quercetin (9.69) (Table 7). Visual color of dyed cotton clearly showed that the color of cotton dyed with hydrolyzed extract fell in the color range of cotton dyed with Quercetin standard or the untreated Flos Sophorae extract (Tables 8, 9). The color was very different from the color of cotton dyed with Rutin standard (Table 9).

UV protection of cotton dyed with hydrolyzed Flos Sophorae extract

Mean UPF value of the cotton dyed with hydrolyzed extract (12.46) was higher than that of the cotton dyed with the untreated extract (10.19) (Table 10). It was even higher than that of the cotton dyed with Quercetin (10.41) (Table 11). This might be due to the synergistic UV protective effect of rutin compound that remained unhydrolyzed in the Flos Sophorae extract. The highest UPF value (13.80) was obtained in the sample dyed with the hydrolyzed FS:DW 75:25 for 60 min (13.80) followed by the sample dyed with the hydrolyzed FS:DW 50:50 for 60 min (13.57) with less than 1.67% difference. The t-test between the two groups showed a p-value of 0.428 at 0.05 confidence level indicating that the two groups were not statistically different (Table 12). The result implies that higher concentration dyebath and longer dyeing time can result in cotton with higher UV protection. It is also possible that dyeing time has slightly higher effect on the UPF than the concentration of dyebath given the concentration levels tested in this research. The UVA and UVB transmittance of this cotton was 7.96% and 8.52% respectively. This indicated that the dyed cotton blocked 92.04% of UVA and 91.48% of UVB. The sample had a slightly higher efficiency in the protection from UVA than UVB. Such result was in good agreement with Choquenet et. al. (2008) who showed that quercetin had slightly

Table 6 Color values of cotton dyed with untreated and hydrolyzed extract of Flos Sophorae

Dyebath conc	Dyeing time	Untreated Flos Sophorae extract					Hydrolyzed Flos Sophora extract				
		L^*	a^*	b^*	ΔE	K/S	L^*	a^*	b^*	ΔE	K/S
Undyed		91.82	3.07	− 13.94	0.00	n.a	90.34	2.87	− 13.10	0.00	
50:50 FS:DW	30 min	80.83	0.09	59.01	73.84	6.16	78.18	4.32	63.75	87.69	9.15
	60 min	79.70	1.86	59.60	74.55	6.06	77.21	5.12	62.06	83.33	11.11
75:25 FS:DW	30 min	80.35	− 0.72	59.98	74.94	6.29	80.76	0.56	50.09	79.09	9.19
	60 min	79.29	0.94	59.24	74.29	6.19	79.79	1.27	52.19	76.39	9.72
Mean		80.04	0.54	59.46	74.41	6.18	78.99	2.82	57.02	81.63	9.79

Table 7 Color values and color strength values of cotton dyed with Rutin and Quercetin standards

Dye conc	Dyeing time	Rutin standard					Quercetin standard				
		L^*	a^*	b^*	ΔE	K/S	L^*	a^*	b^*	ΔE	K/S
Undyed		90.34	2.87	− 13.1	0.00	n.a	90.34	2.87	− 13.1	0.00	
5%	30 min	87.94	− 6.28	26.07	40.31	4.61	80.45	− 2.38	73.67	87.50	8.09
	60 min	87.64	− 6.23	27.84	42.03	4.43	74.58	− 1.16	73.18	87.83	11.50
10%	30 min	86.13	− 6.49	36.12	50.30	4.60	78.92	− 5.13	72.41	86.64	9.84
	60 min	87.01	− 6.56	37.35	51.44	4.47	79.53	− 5.16	72.05	86.25	9.32
Mean		87.18	− 6.39	31.85	46.02	4.53	78.37	− 3.46	72.83	87.06	9.69

Table 8 Photographs of cotton dyed with untreated and hydrolyzed extract of Flos Sophorae

Dyebath conc.	Dyeing time	Untreated Flos Sophorae extract			Hydrolyzed Flos Sophora extract		
		Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
50:50 FS:DW	30 min						
	60 min						
75:25 FS:DW	30 min						
	60 min						

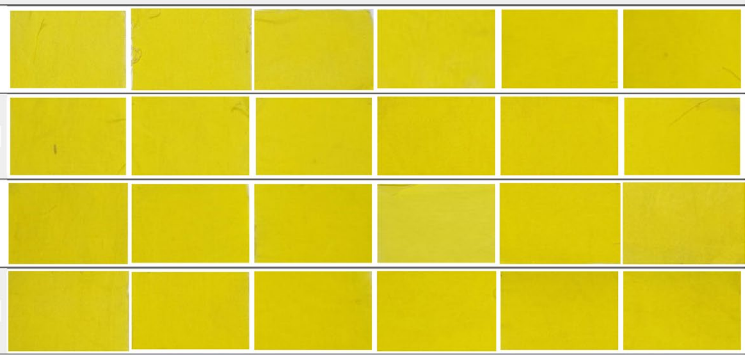
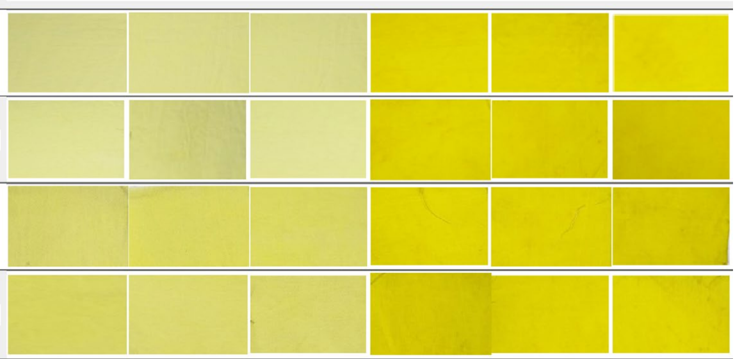


Table 9 Photographs of cotton dyed with Rutin and Quercetin standards

Dye conc.	Dyeing time	Rutin standard			Quercetin standard		
		Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
5%	30 min						
	60 min						
10%	30 min						
	60 min						



higher effectiveness in filtering UVA than UVB when quercetin and rutin were examined of their SPF and PF-UVA values.

Independent t-test was conducted between the mean UPF values of the cotton dyed with different dyebaths (Table 12). The statistical significances were examined at 0.05 confidence level. There was a significant difference ($p = 0.000$) between the undyed cotton and the cotton dyed with the hydrolyzed extract of FS 50:50 30 min (which gave

Table 10 UV protection values of cotton dyed with untreated and hydrolyzed extract of Flos Sophorae

Dyebath conc	Dyeing time	Untreated Flos Sophorae extract				Hydrolyzed Flos Sophora extract			
		UPF		Transmittance (%)		UPF		Transmittance (%)	
		Mean	SE	UVA	UVB	Mean	SE	UVA	UVB
Undyed		6.57	0.21	17.72	18.22	6.57	0.21	17.72	18.22
50:50 FS:DW	30 min	10.00	0.15	9.34	9.92	10.84	0.03	9.67	10.07
	60 min	9.70	0.11	9.27	9.76	13.57	0.90	8.51	9.25
75:25 FS:DW	30 min	10.74	0.18	9.13	9.61	11.63	0.23	9.69	10.02
	60 min	10.30	0.11	9.32	9.83	13.80	0.74	7.96	8.52
Mean		10.19	0.14	9.27	9.78	12.46	0.48	8.96	9.47

Table 11 Mean UV protection values of cotton dyed with Rutin and Quercetin standards

Dye conc	Dyeing time	Rutin standard				Quercetin standard			
		UPF		Transmittance (%)		UPF		Transmittance (%)	
		Mean	SE	UVA	UVB	Mean	SE	UVA	UVB
Undyed		6.57	0.21	17.72	18.22	6.57	0.21	17.72	18.22
5%	30 min	8.03	0.23	9.32	9.71	10.53	0.29	9.93	10.69
	60 min	7.27	0.31	9.42	9.89	10.78	0.88	9.89	10.65
10%	30 min	7.78	0.11	9.79	10.44	10.08	0.41	9.72	10.47
	60 min	8.23	0.37	9.82	10.51	10.23	0.46	9.70	10.45
Mean		7.83	0.26	9.59	10.14	10.41	0.35	9.81	10.57

Table 12 Results of independent t-test on the UPF values

No	Compared groups	N	UPF			
			Mean	SE	*t*	*p*
1	Undyed	3	6.57	0.21	− 20.343*	0.000
	Hydrolyzed FS 50:50 30 min	3	10.84	0.03		
2	Undyed	3	6.57	0.21	− 9.355*	0.001
	Hydrolyzed FS 75:25 60 min	3	13.80	0.74		
3	Untreated FS 75:25 30 min	3	10.74	0.18	− 3.998*	0.016
	Hydrolyzed FS 75:25 60 min	3	13.80	0.74		
4	Rutin 10% 60 min	3	8.23	0.37	− 6.708*	0.003
	Hydrolyzed FS 75:25 60 min	3	13.80	0.74		
5	Quercetin 5% 60 min	3	10.78	0.88	− 2.614	0.059
	Hydrolyzed FS 75:25 60 min	3	13.80	0.74		
6	Hydrolyzed FS 50:50 60 min	3	13.57	0.90	− 0.195	0.428
	Hydrolyzed FS 75:25 60 min	3	13.80	0.74		

the lowest UPF among the hydrolyzed samples). A significant difference ($p=0.001$) was also found between the undyed cotton and the cotton dyed with the hydrolyzed extract of FS 75:25 60 min (which gave the lowest UPF among the hydrolyzed samples). Cotton with the highest UPF values of the hydrolyzed dyeing group and the other 3 dyeing groups- untreated Flos Sophorae, Rutin and Quercetin standards- were examined in regard to the statistical significance. There were significant differences in the UPF values between the cotton dyed with hydrolyzed extract vs. untreated extract ($p=0.016$), and between the cotton dyed with the hydrolyzed extract vs. Rutin ($p=0.003$). However, the UPF values of cotton dyed with the hydrolyzed extract and the cotton dyed with Quercetin were not significantly different ($p=0.059$). This indicated that cotton dyed with the hydrolyzed extract of Flos Sophorae gave UV protection comparable to and higher than that of cotton dyed with Quercetin standard.

Effect of washing and sunlight on the UPF and the colorfastness

Cotton samples dyed with the hydrolyzed extract of Flos Sophorae were exposed to xenon light for 40 h and were subject to washing according to the standard tests of colorfastness to sunlight and colorfastness to washing (Table 13). The difference in the UPF value before and after the colorfastness test was the greatest in the cotton dyed with 75:25 FS:DW dyebath for 60 min. The UPF value of this sample decreased 12.4% after xenon light exposure and decreased 26.88% after washing. The least difference in the UPF value before and after the colorfastness test was observed in the cotton dyed with 50:50 FS:DW dyebath for 30 min. The sample showed a 0% loss of UPF after sunlight exposure and 6.09% increase in the UPF after washing. The samples showed 4–5 grades for colorfastness to sunlight and 3–4 grades for the colorfastness to washing (Table 14).

Discussion of results

The results of present investigation showed that higher color strength value was generally related to the higher UPF value (Fig. 9). The ΔE^{*} value was higher and the b* value for yellowness was only slightly different than the cotton dyed with untreated Flos Sophorae extract. These results were quite different from Gorjanc et. al. (2014) who reported that dyeing cotton in the presence of UV absorber resulted in a substantial color change to a lighter color. Such result was due to the competition of UV absorber and the dye in the dyebath both of which needed to react with the surface of cotton fiber (Gorjanc et al., 2014).

Since quercetin is a pigment and at the same time it has a UV protective effect there would be no such competition when dyeing cotton with the hydrolyzed extract of Flos Sophorae extract. This was evidenced by the increase in the K/S value as well as the UPF value when cotton was dyed using the hydrolyzed extract of Flos Sophorae compared to dyeing with the untreated Flos Sophorae extract. Dyeing with the hydrolyzed extract of Flos Sophorae did not alter the expected yellow color of Flos Sophorae dyeing. By converting rutin compound to quercetin compound in natural Flos Sophorae extract the UV protection effect of cotton dyed by this extract can be enhanced by affixing quercetin instead of rutin to the fabric (Zhou & Tang, 2017).

The UPF values obtained from the cotton dyed with hydrolyzed extract was in the range of 10.84–13.80. These values are lower than the UPF value which can be labeled on the garment that is designed for the purpose of UV protection (Gorjanc et al., 2014; Narantuya & Ahn, 2020; Sungrubbies, 2022; "Sun protective clothing," 2021). However, by dyeing with hydrolyzed Flos Sophorae extract the UPF value of the light-weight summer cotton was enhanced to 13.80 which indicated 92.76% blockage of the UV rays as

Table 13 UPF of cotton dyed with the hydrolyzed extract of Flos Sophorae before and after the fastness tests

Dye conc	Dyeing time	Before			After sunlight fastness test				After washfastness test			
		UPF	Transmittance (%)		UPF		Transmittance (%)		UPF		Transmittance (%)	
			UVA	UVB	UPF	% Loss	UVA	UVB	UPF	% Loss	UVA	UVB
50:50 FS:DW	30 min	10.84	9.67	10.07	10.84	0.00	11.83	10.51	11.50	−6.09	8.99	9.72
	60 min	13.57	8.51	9.25	11.58	14.66	11.63	10.32	11.84	12.75	9.80	10.56
75:25 FS:DW	30 min	11.63	9.69	10.02	11.31	2.75	11.51	10.21	10.87	6.53	9.17	9.86
	60 min	13.80	7.96	8.52	12.24	11.30	11.29	10.02	10.09	26.88	9.27	9.76

Table 14 Colorfastness grades of cotton dyed with hydrolyzed extract of Flos Sophorae

Dye conc	Dyeing time	Colorfastness to sunlight	Colorfastness to washing
50:50 FS:DW	30 min	4	3
	60 min	4–5	3–4
75:25 FS:DW	30 min	4–5	3–4
	60 min	4–5	4

opposed to 84.78% blockage by the same fabric without dyeing. By dyeing, the percent blockage of UVA and UVB became 92.04% and 91.48% respectively showing 2.23 times and 2.14 times increase of protection compared to the same cotton fabric without dyeing. Based on the results it is possible to suggest that the present dyeing method could produce light-weight summer cotton with yellow color which could provide a moderate UV protection for daily summer attire. Additionally, it is to be reminded that the HCl concentration that was used for the hydrolysis of Flos Sophorae extract for dyeing the cotton fabric was 0.5 M which showed the relative quercetin yield of 52.03% whereas with 1 M HCl quercetin yield was 93.09%. Higher UPF value can be obtained on the same cotton fabric when 1 M HCl concentration is used for the hydrolysis of actual dyeing process.

Dyeing mechanism of hydrolyzed Flos Sophorae extract and more specifically that of quercetin on cotton is based on the formation of fiber-metal-dye chelation by the alum mordanting that was simultaneously done during dyeing (Samanta & Konar, 2011). The aluminum ion of potassium alum ($KAl(SO_4)_2 \cdot 12H_2O$) that is bonded to the fiber will attract quercetin so that the dye can indirectly bond to the fiber via the fiber-Al-quercetin link (Samanta & Konar, 2011). Ilmi et. al. (2020) suggested that $AlCl_3$ formed a complex with quercetin on the keto groups of C4 atom and the neighboring hydroxyl groups at C3 and C5 atoms. Similar anchoring position of aluminum to quercetin is probable in the alum mordanting of Flos Sophorae dyeing.

Conclusions

This research investigated the method of acid hydrolysis of Flos Sophorae extract to convert rutin in the extract to quercetin for the purpose of enhancing the UV protection of cotton dyed thereof. It was found that the hydrolysis using the mixed solution of methanol, 1 M HCl, and water gave excellent result for the conversion of rutin to quercetin. Dyeing cotton using the Flos Sophorae extract hydrolyzed with 0.5 M HCl more than doubled the UPF value of the undyed cotton compared to the cotton dyed without the hydrolysis treatment. The increased UV protection of cotton dyed with the hydrolyzed Flos Sophorae extract was based on the excellent UV absorbance characteristics of quercetin. The UPF value obtained from dyeing cotton with the hydrolyzed Flos Sophorae extract was lower than the UPF required for labeling the garment with the so-called UV protective garment. However, the present dyeing method could produce light-weight summer cotton with yellow color which could provide a moderate UV protection for daily summer attire. Further research would be necessary to conserve the UPF value

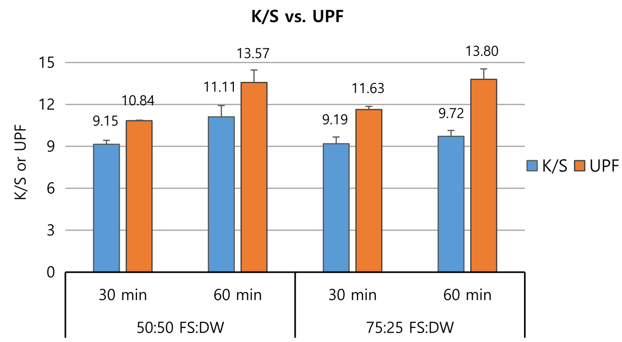


Fig. 9 Comparison of color strength (K/S) and UV protection

upon sunlight exposure and washing. This research was the first attempt to apply the hydrolysis method for Flos Sophhorae dyeing for the purpose of enhancing the UV protection of a light-weight summer cotton fabric.

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IRB permission number: None. Research deals with cotton fabric and dried plant material sold in the market. It does not deal with human research subjects.

Author contributions
The data presented in this manuscript is part of the Doctoral dissertation of LN. LN designed the research, conducted all the experiments, collected and analyzed the data. CS conducted part of the data analyses, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declaration

Competing interests
The authors declare that they have no competing interests.

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