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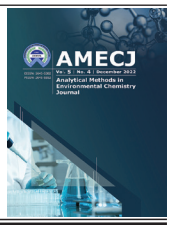

In-vitro evaluation of photoprotection, cytotoxicity and phototoxicity of aqueous extracts of Cuscuta campestris and Rosa damascene by MTT method and UV spectroscopy analysis

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ABSTRACT

Applying sunscreen is essential for protecting the skin from UV's acute and chronic effects. Some of these products on the market display side effects and are expensive. There is a great demand for effective, cheap, safe, and herbal sunscreens with a wide range of sun protection activities. This study aimed to evaluate the photoprotection, cytotoxicity, and phototoxicity of aqueous extracts of Cuscuta campestris (CC-AE) and Rosa damascena (RD-AE). The maceration method prepared the CC-AE and RD-AE from the aerial branch. In-vitro photoprotection was evaluated by determining the sun protective factor (SPF) of CC-AE and RD-AE by a UV-visible spectrophotometer. The cytotoxicity and phototoxicity studies were assessed using the MTT assay on 3T3 cells. In the final, the PIF (Photo Inhibitor Factor) was calculated. The SPF values of CC-AE and RD-AE were found at 11.10±0.05 and 1.36±0.04, respectively, at the concentration of 0.2 mg mL^{-1}. The half maximal effective concentration (EC_{50}) of CC-AE and RD-AE was obtained at 35.05±0.91 µg mL^{-1} and 40.7±0.87 µg mL^{-1}, respectively. The phototoxicity analysis showed that CC-AE and RD-AE had low PIF values and were considered as the probable phototoxic. Overall, regarding the considerable SPF and PIFs values plus the anti-inflammatory and antioxidant properties of these extracts, they can be evaluated for further pharmaceutical formulations.

1. Introduction

Solar ultraviolet (UV) radiation such as UVA (320–400 nm) and UVB (~295–320 nm) have acute and chronic influences on the skin; they might finally cause cancers of the skin [1]. UVB radiation can cause acute effects such as erythema and edema, and chronic effects such as immunosuppression and carcinogenesis [2, 3]. However, UVA radiation can induce tanning by the oxidation of melanin, and photoaging by the destruction of dermal structures, as well as leading to damage of the macromolecules, and oxidative stress by the production of reactive oxygen species (ROS) [2, 3]. The main destructive factors of UV radiation on the skin are free radicals including superoxide anions, hydroxyl radicals, singlet oxygen,

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hydrogen peroxide, ferric ion, nitric oxide, etc. [3]. Photoprotection which is caused with using of sunscreen, prevents the acute and chronic effects of UV radiation. UV protectors are classified as UV filters and UV absorbers based on types of cosmetic materials [2, 4]. UV filters are divided into two classes according to their chemical structure and mechanism of action: inorganic, such as titanium dioxide and zinc oxide, are of low irritation potential and exhibit photostability and wide-ranging absorption spectra and organic such as UVA, UVB, and broadband absorbers that absorb the radiations based on their chemical structure. The filter's ability of organic filters is classified as a photostable, photo-unstable, and photoreactive filters [2, 4, 5]. The sunscreens' formulations that protect the skin from harmful UV rays could be introduced as physical and chemical sunscreens by blocking, reflecting, scattering and absorbing the UV rays [2, 5]. The efficacy rate of sunscreens is usually measured by the sun protection factor (SPF) estimation, which represents an accepted global characteristic of protection from erythema after exposure to simulated solar radiation [6]. Generally, the components of sunscreens have shown side effects such as disruption in the endocrine system and changes in the hypothalamic-pituitary-thyroid (HPT) axis. In addition, they could be caused reproductive homeostasis during long-term use [4, 7]. However, some sunscreens may have environmental toxicity effects and can have detrimental effects on the ecosystem [2, 4]. Best sunscreens should have several characteristics, including safe, non-toxic, and photo-stable, and be able to protect the skin from UVA and UVB rays [4]. The natural photoprotectants can be included the obtained extracts of plants such as aloe vera, pomegranate, rambutan, grape, tomato, the green tea, and the oils obtained from soybean, olive, coconut, almond, and jojoba as well as the mycosporine-like amino acids (MAA), etc. [5, 8-10]. Several studies have described the use of plant extracts with photoprotection properties. For example, Rangel et al [2] assessed the photoprotective capability of extracts from red macroalgae. Permana et al [11] showed a potential absorption of UVA and UVB radiation by the hydrogel-containing propolis extract-loaded phytosome and indicated their high SPF value of them [11]. Natural combinations have shown the desirable SPF and anti-inflammatory and antioxidant properties [9, 12]. Rosa damascena mill, commonly known as Gole Mohammadi in Iran [13], showed several medicinal properties including antiviral, antimicrobial, antioxidant, antitussive, hypnotic, anti-diabetic, and sedative effects on the respiratory system [14]. This plant contains different chemical compounds such as tannins, polyphenols, carotenoids, quercetin, eugenol, citronellol, geraniol, liquiritin, etc. [14, 15]. Generally, the extracts of rose petals have shown high antioxidant activity that correlated to the total phenolic, and flavonoid contents of rose [16, 17]. The analgesic and anti-inflammatory effects of rose have also been reported [18-20]. The hydroalcoholic extract of R. damascene can significantly reduce edema, which may be mediated by the inhibition of acute inflammation [13]. Cuscuta campestris Yuncker with the common name dodder has analgesic, antipyretic, anti-inflammatory, and anti-cancer properties [21, 22]. This holoparasitic plant has been applied to treat a liver injury, cancer prevention, sciatica, scurvy, and scrofula derma [22-24]. Based on reported works, polyphenolic compounds such as quercetin, sinapic acid, kaempferol, isorhamnetin hesperidin, and eugenol were identified in extracts from C. campestris [25, 26]. The ethyl acetate extract of the plant has the strongest antioxidant effect due to the highest content of flavonoid compounds kaempferol and quercetin [22]. A review of the literature did not expose any previous studies on the photoprotective, cytotoxicity, and phototoxicity activities of the aqueous extract of Cuscuta campestris (CC-AE) and Rosa damascena (RD-AE) plants by MTT method and UV spectroscopy analysis. Generally, the UV/visible spectrophotometric method were applied to analyze the UV radiation protection capability for probable sunscreen applications [2, 6]. One of the most important issue in pharmaceutical circles is to optimizing the wright method for analyzing the active ingredients in bulk drug materials, their impurities and decompositions substances, and also pharmaceutical formulations and biological

products. Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. The use of UV-Vis spectrophotometry, especially in the analysis of pharmaceutical forms, has increased rapidly in recent years [2, 3, 26]. In vitro methods for evaluating the sunscreen potentials of materials are generally of two types. Methods that involve measuring the absorption or transmission of UV radiation through sunscreen product films on quartz plates or bio-membranes, and methods in which the absorption characteristics of sunscreen agents are determined based on spectrophotometric analysis of dilute solutions [2, 3, 26].

In the present study, the UV absorption of each sample was obtained and the Mansur equation was applied to find the final SPF. Afterwards the effects of extracts were evaluated in vitro in 3T3 cells to obtain their probable photo-toxic or photo-protective behaviors.

2. Materials and Methods

2.1. Chemicals

Trypsin, phosphate-buffered saline (PBS), and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) were supplied by Sigma company (St. Louis, MO, USA). Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), and Penicillin-Streptomycin solution (100X) were obtained from Borna Pouyesh Gene Company (BPGene Co., Kerman, Iran).

2.2. Extracts preparation

The plants (C. Campestris and R. Damascena) were collected from Mahan, Kerman, Iran (30.0630° N, 57.2875° E). The plants were then identified by Dr. Mitra Mehrabani and kept in the Faculty of Pharmacy herbarium (Kerman University of Medical Sciences, Kerman, Iran). The aerial branches of plants were washed three times with deionized water and dried at room temperature. The dried aerial branches were ground with a mill to obtain a fine powder. The extracts of plants were prepared using the maceration method. For this purpose, 10 g of the fine powder was combined with the deionized water (100 mL) in a laboratory flask with a volume of 500 mL. The mixture was heated at 80 °C for 30 min and filtrated through Buchner funnel linked with Watman filter paper (No.1). Finally, the filtrate was freeze-dried (freeze dryer FD-550 purchased from Tokyo Rikakikai Co., Ltd, Japan) [27]. The dried aqueous extract of C. campestris and R. damascena were labeled as CC-AE and RD-AE, respectively, and the SPF of compounds was determined by UV-visible spectrophotometer.

2.3. Determination of UV absorption spectra by UV-vis spectrophotometer

The characterizations of UV absorption spectra were carried out by analyzing an aqueous extract of C. campestris and R. damascena at concentrations 20000, 10000, 5000, 2500, and 1250 µg mL^{-1}. The UV spectra were recorded using a Synergy TM 2 multi-mode microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) from 200 to 900 nm.

2.4. Determination of photoprotection activity of plants extracts

The procedure of Khazaeli and Mehrabani [28], with some modifications, was used to measure the photoprotection activity of plant extracts. For this purpose, the obtained aqueous extracts of C. campestris and R. damascene were individually scanned in the range from 337.5 nm to 292.5 nm with interval five nm using a double beam UV/Vis spectrophotometer (Optizen 3220 UV). Then, in vitro SPF was measured by the following equation I [29].

$$SPF = \frac{\sum_{292.5}^{337.5} E(\lambda).\varepsilon(\lambda)}{\sum_{292.5}^{337.5} E(\lambda).\varepsilon(\lambda).T(\lambda)} \qquad \text{(Eq.I)}$$

Where T(λ), E(λ), and ε(λ) represents the transmittance of the sample at λ, the spectral irradiance of terrestrial sunlight at λ, and the erythemal action spectrum at λ, respectively. The E(λ) × ε(λ) values are showed in Table 1, the T(λ) was three times measured and the obtained means were applied to estimate the SPF value for each extract. Afterward, the graph relationship of SPF versus LnC was used to calculate SPF in 2.0 mg mL^{-1} solution for each extraction.

Table 1. Normalized product function used in the calculation of SPF.

Wavelength (nm)	E(λ) × ε(λ)
292.5	1.139
297.5	6.510
302.5	10.00
307.5	3.577
312.5	0.973
317.5	0.567
322.5	0.455
327.5	0.289
332.5	0.129
337.5	0.046

E(λ): the spectral irradiance of terrestrial sunlight at each wavelength.
ε(λ): the erythemal action spectrum at each wavelength.

2.5. Cell culture

The mouse embryonic fibroblast cells (3T3) (ATCC Number: IBRCC10100) were provided by the Iranian Biological Resource Center (IBRC) in Tehran, Iran. The cell line was cultured in DMEM medium supplemented with 10% (v/v) FBS, 100 U mL^{-1} penicillin, and 100 µg mL^{-1} streptomycin and incubated at 37 °C in a 5% CO_2 incubator [30].

2.6. Cytotoxicity assay

Based on methods reported in the literature [31-34], in the exponential growth stage, the cells were harvested and seeded into 96-well tissue culture plates (approximately 10^4 cells per well). After 24 h, the samples of serial concentrations of CC-AE and RD-AE (at the final concentration range of 3.9–125 µg mL^{-1}) were separately poured into the desired wells. After 24 h, the medium in each well was switched with 20 µL of MTT solution (5 mg mL^{-1}) and plates were incubated at 37 °C for a further 3 h. For dissolving the formazan crystals, the culture media were removed from the wells and 100 µL of fresh DMSO was added to each well of the plate. The optical density of final solutions was then read at 570 nm using a Synergy ™ 2 multi-mode microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). Doxorubicin (12 µg mL^{-1}) was applied as a positive control. All experiments were repeated in triplicate on different days and EC_{50} values were determined and analyzed by non-linear regression analysis (SPSS software, SPSS inc., Chicago) and the data were reported as mean (m±SD).

2.7. Evaluation of phototoxicity and determination of PIF factor

For the purpose of evaluation of phototoxicity in the presence and absence of UVA radiation [35], cells were prepared as described in the cytotoxicity assay into two plates (A and B). Plate A was exposed to UVA light (1.8 mW cm^{-2}) for 60 min. After 60 min, the medium was discarded and the fresh medium was added. Plate B was used as a non-irradiated control. Both plates were incubated for 24 h at 37 °C in a 5% CO_2 incubator. Afterward, the medium in each well was discarded and MTT solution (20 µL, 5 mg mL^{-1}) was added. Plates were incubated at 37 °C for 3 h and following the culture, media were removed from the wells and 100 µL of fresh DMSO was added to each well to dissolve the formazan crystals. The absorption was then measured at 570

nm, and the EC_{50} values were estimated. The PIF (Photo Inhibitor Factor) was determined based on below equation II:

$$PIF = \frac{EC_{50(-UV)}}{EC_{50(+UV)}} \qquad \text{(Eq.II)}$$

In compliance with the OECD TG 432 [36], the below Table was considered for analyzing the PIF values (Table 2).

Table 2. The categorization of phototoxicity stages based on PIF values.

PIF value	Type of hazard
PIF < 2	Non phototoxic
PIF > 2 and < 5	Probable phototoxic
PIF > 5	Potential phototoxic

2.8. Investigation of protective effects of plant extracts against of phototoxic effects of chlorpromazine

In assessing of the ability of plant extracts to prevent of the phototoxic effects of chlorpromazine (CPZ), two culture plates (A and B) were seeded with about 10^4 cells per well. Then, the CC-AE and RD-AE were prepared at a concentration of 31.25 µg mL^{-1}. The concentrations of chlorpromazine were also trained at the range of 0.1, 0.5, and 1 µg mL^{-1}. After 24 h, the culture media on the cells were evacuated and 100 µL of the prepared concentration of extracts and 100 µL of the concentrations of chlorpromazine were separately added into the desired wells of the culture plates. Subsequently, the plate A was exposed to UVA light (1.8 mW cm^{-2}) for 60 min. Over time, the cultural media were removed and the fresh media were added. The plate B was maintained in darkness (as a non-irradiated control). The culture plates (A and B) were incubated at 37 °C for 24 h in a 5% CO_2 incubator. The subsequent steps were performed as in Section 2.8. All experiments were repeated three times in different days. Then, the cell viabilities (%) were determined, and data were stated as mean results (m±SD).

2.9. Statistical analysis

Experimental data are presented as the mean (m±SD) with at least three determinations for independent experiments. All data were analyzed by non-linear regression analysis (SPSS software, SPSS inc., Chicago) and the p-valve ($p< 0.05$) was considered to be statistically significant.

3. Results and Discussion

3.1. UV absorption spectra and critical wavelength

The UV absorption spectra of CC-AE and RD-AE are shown in Figure 1. The max absorbance of CC-AE (at 2500 µg mL^{-1}) and RD-AE (at 2500 µg mL^{-1})

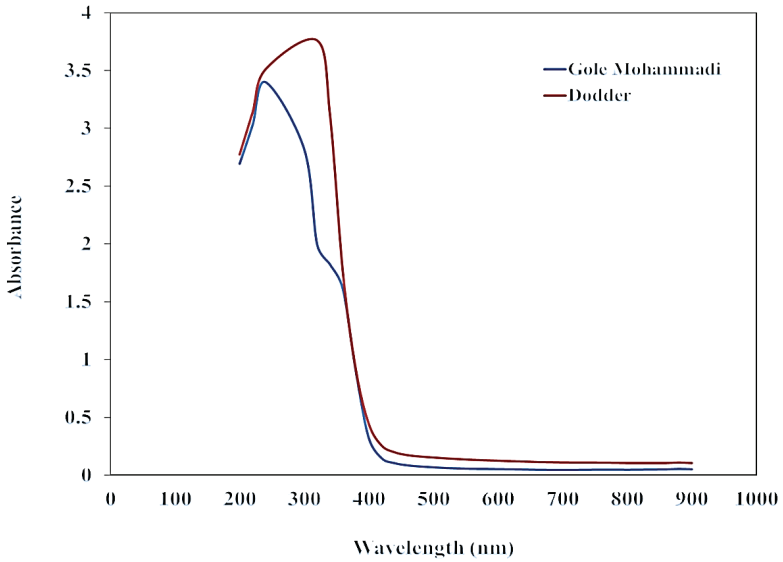

Fig. 1. The UV absorption spectra of aqueous extracts of Rosa damascena and Cuscuta campestris at concentration 2500 µg mL^{-1}

were at 240 nm and 320 nm, respectively (Fig. 1).

3.2. In vitro SPF assessment by UV Spectrophotometry analysis

The SPF is a quantitative capacity of the efficiency of a sunscreen product. To prevent sunburn and other skin damage, a sunscreen product should have a broad absorption of between 290 and 400 nm. Antioxidants from natural resources, especially plants, might be offered as novel potentials for the treatment and prevention of diseases caused by UV rays. There are reports on the correlation between antioxidant activity and SPF values [2, 37]. Based on previous reports of the excellent antioxidant activity of CC-AE and RD-AE plants [16, 17, 25], the current study investigated the SPF values of aqueous extracts of plants by UV spectrophotometry applying Mansur mathematical equation [6]. In Table 3, the SPF values measured using the UV transmission spectra of CC-AE and RD-AE are listed. As shown in Table 3, the SPF values obtained at 2 mg mL^{-1} were 11.10±0.05 and 1.36±0.04 for CC-AE and RD-AE, respectively. Ebrahimzadeh et al [38] assessed the SPF values of extracts from Sambucus ebulus, Zea maize, Feijoa sellowiana, and Crataegus pentagyna and reached the highest value (SPF = 24.47) using ultrasonic extract of Crataegus pentagyna. They also reported that there is a good correlation between SPF and phenolic contents. Hashemi et al [37] reported the highest SPF values (0.841 and 0.717) for Cucumis melo leaf ultrasonic extract and Artemisia absinthium shoots methanolic extract, respectively. Da Silva Fernandes et al [36] obtained a low SPF (2.5±0.3) for an aqueous fraction (AF) from Antarctic moss Sanionia uncinata; however, the SPF values increased more than three times in association with UV-filters with AF. The highest value (25.8±0.3) was reported in AF plus 3-(4 methylbenzylidene)-camphor [36]. In another study, the sunscreen formulations prepared by using the combination of organic UV filters (w/w %), and Olea europaea leaf extract (OLE, w/w %) and measured in vitro photoprotective efficacy using a UV transmittance analyzer for the determination of SPF values [7]. The SPF values 56±3, 42± 5, and 21±2 were obtained by formulations that contained 5%, 3%, and 1% OLE, respectively [7]. Therefore, the association of UV filters with different plant extracts can be increased the efficiency of sunscreen formulations [7, 36].

3.3. Phototoxicity Analysis

The toxicity effects of the CC-AE and RD-AE on the 3T3 cell line were analyzed using the MTT-based colorimetric test after 24 h; however, phototoxicity was evaluated by comparing the difference in toxicity between the sample plate that was not exposed to UVA light and the sample plate exposed to UV light. The half-maximal effective concentration (EC_{50}), without UVA light, for 3T3 cell line treated with CC-AE, RD-AE, and was measured to be 35.05±0.91 µg mL^{-1}, 40.7±0.87 µg mL^{-1}, and 16.79±0.35 µg mL^{-1}, respectively (Table 4). According to analyses of

Table 3. Calculation of SPF of the aqueous extracts of plants in different concentrations by UV–visible spectrophotometry

Plant	Concentration of aqueous extract (µg mL^{-1})	SPF[a]
Cuscuta campestris	10	0.070±0.04
	50	2.000±0.05
	500	7.450±0.04
	2000	11.10±0.05
Rosa damascena	10	0.027±0.04
	50	0.110±0.05
	500	1.072±0.05
	2000	1.360±0.04

[a] Data represent means±SE (n=3).

the PIF, CC-AE (PIF=3.55) and RD-AE (PIF=2.35) were exhibited as probable phototoxic in the tested doses (Table 2). Chlorpromazine (PIF=35.59) was a potential phototoxic hazard and results were obtained for the cell viability with a difference approximately 35-fold in EC_{50} values, with and without UV light (Table 4). Amaral et al [39] presented that the IC_{50} values for Caryocar brasiliense supercritical CO_2 extract (CBSE) in the phototoxicity assay considered 6.50% w/v in dark conditions and 35.53% w/v in irradiated conditions. According to the PIF value, the CBSE not exhibited phototoxic potential (PIF=0.18). Da Silva Fernandes et al [36] reported that the AF presented non-phototoxic (PIF=1.089) and the AF in mixtures with UV filters did not offer any phototoxic potential (PIF < 2). Svobodová et al [40] assessed the phototoxic potential of silymarin, an identical extract of the seeds of Silybum marianum, and its bioactive components. The obtained results showed that silymarin and its major component had no phototoxicity. Nathalie et al [35] assessed the phototoxic of some essential oils and showed that the PIF values of lemongrass oil, orange oil, and CPZ were 2.34, 2.21, and 31.24, respectively, as probably phototoxic hazard by 3T3/MTT procedure [35]. Consequently, in the present study, C. campestris and R. damascene aqueous extracts can be identified as probable phototoxic ingredients; however, additional investigations are needed to evaluate the health risks associated with them in vivo.

3.4. Analysis and Evaluation of protective effects of plant extracts on prevention of phototoxic effects of chlorpromazine

The effect of combinations of C. campestris aqueous extract, and/or R. damascena aqueous extract and chlorpromazine as strong phototoxic substance were assessed using the MTT-assay on the 3T3 cell line. These experiments were evaluated using the combination of a concentration of CC-AE or RD-AE (31.25 µg mL^{-1}) with three concentrations of CPZ (0.1, 0.5 and, 1 µg mL^{-1}) in the presence and absence of UVA light. The obtained results of cell viability (%) are shown in Table 5. After 24 h, the measured cell viabilities (%) for the 3T3 cell line treated with a combination of the CC-AE and the different ranges of CPZ were 53.70±1.51%, 49.15±1.01%, and 43.67±1.2%, respectively, in the absence of UVA light; however, the measured cell viabilities (%) were 49.59±2.00%, 45.44±1.51%, and 37.47±0.93%, for similar concentrations, in the presence of UVA light (Table 5). The measured cell viabilities (%) for the studied concentration of RD-AE on the different concentrations of CPZ were 51.29±1.13%, 46.43±1.64%, and 41.82±0.86, respectively, in the absence of UVA light. Measured cell viabilities were 43.36±1.02%, 35.53±1.33%, and 47.78±2.1%, respectively, in the presence of UVA light (Table 5). Generally, in the fact of UVA light, the measured cell viabilities of CPZ alone were lower than the combination of CC-AE and CPZ. The measured cell viabilities of CPZ alone at concentrations 0.5 µg mL^{-1} and 1 µg mL^{-1} were higher than the combination of RD-AE and CPZ (Table 5).

Table 4. Evaluation of the cytotoxicity and phototoxicity of the aqueous extracts of plants and chlorpromazine in murine fibroblasts cell (3T3)

Sample	UV radiation[a]	EC_{50}[b]	PIF
Cuscuta campestris aqueous extract (CC-AE)	-	35.05±0.91	3.55
	+	9.86±0.61	
Rosa damascena aqueous extract (RD-AE)	-	40.7±0.87	2.35
	+	17.31±0.22	
Chlorpromazine (CPZ)	-	16.79±0.35	35.59
	+	0.467±0.06	

[a] – or + represents the tests performed with and without UV light.
[b] Data represent the mean±SD of three experiments in different days

Table 5. Evaluation of protective effects of plant extracts on prevention of phototoxic effects of chlorpromazine in murine fibroblasts (3T3).

Plant	CAE* (μg mL^{-1})	Chlorpromazine (μg mL^{-1})	UV radiation [a]	Cell viability [b] (%)
Cuscuta campestris	31.25	1	-	53.70±1.51
	31.25	0.5	-	49.15±1.01
	31.25	0.1	-	43.67±1.20
	31.25	1	+	49.59±2.00
	31.25	0.5	+	45.44±1.51
	31.25	0.1	+	37.47±0.93
Rosa damascena	31.25	1	-	51.29±1.13
	31.25	0.5	-	46.43±1.64
	31.25	0.1	-	41.82±0.86
	31.25	1	+	43.36±1.02
	31.25	0.5	+	35.53±1.33
	31.25	0.1	+	47.78±2.10
Chlorpromazine	-	1	-	55.22±1.09
	-	0.5	-	53.60±2.11
	-	0.1	-	53.60±1.21
	-	1	+	47.66±1.21
	-	0.5	+	38.04±0.98
	-	0.1	+	32.86±0.88

* CAE: Concentration of aqueous extract

[a] $^{-}$ or $^{+}$ represents the tests performed with and without UVA light

4. Conclusion

Ultraviolet rays cause numerous injuries to the skin, so there is a vital need to protect it against its harmful effects. Natural materials usually have the ability to protect against the toxic effects of ultraviolet rays. Based on favorable antioxidant and anti-inflammatory properties of Cuscuta campestris (CC-AE) and Rosa damascena (RD-AE) plants, the current study investigated the photoprotection, cytotoxicity and phototoxicity activities of aqueous extracts of CC-AE and RD-AE in mouse fibroblast cells (3T3 cells) by MTT method and UV spectroscopy analysis. In this research, the SPF values of CC-AE and RD-AE were evaluated by UV–visible spectrophotometry applying the Mansur equation. At the concentration of 0.2 mg mL^{-1}, the SPF values of CC-AE and RD-AE were 11.10±0.05 and 1.36±0.04, respectively. The EC_{50} of CC-AE and RD-AE was 35.05±0.91 μg mL^{-1} and 40.7±0.87 μg mL^{-1}, respectively. The PIF values for CC-AE and RD-AE are in the range of probable phototoxic materials (PIF > 2 and < 5), but as these numbers are deficient and near the range of non-phototoxic, they could be hypothesized for future anti-solar formulations. Moreover, in the presence of UVA light, the measured cell viabilities of CPZ alone were lower than the combination of CC-AE and CPZ. Overall, the presented data in this report showed that RD-AE, with SPF and PIF of 11 and 2.35 and various prominent biological effects, could be regarded as an efficient natural product to be considered in sunscreen formulations.

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6. References

[1] S.R. Varma, T.O. Sivaprakasam, I. Arumugam, N. Dilip, M. Raghuraman, K. Pavan, M. Rafiq, R. Paramesh, In vitro anti-inflammatory and skin protective properties of Virgin coconut oil, J. Tradit. Complement. Med., 9 (2019) 5-14. https://doi.org/10.1016/j.jtcme.2017.06.012.

[2] K.C. Rangel, L.Z. Villela, K.d.C. Pereira, P. Colepicolo, H.M. Debonsi, L.R. Gaspar, Assessment of the photoprotective potential and toxicity of Antarctic red macroalgae extracts from Curdiea racovitzae and Iridaea cordata for cosmetic use, Algal Res., 50 (2020) 101984. https://doi.org/10.1016/j.algal.2020.101984.

[3] G. Bhandari, S. Baurai, Assessment of in vitro sun protection factor of plant extracts by ultraviolet spectroscopy method, Univ. J. Phytochem. Ayurvedic Heights, 2 (2020) 20-25. https://doi.org/10.51129/ujpah-2020-29-2(3).

[4] M. Majeed, S. Majeed, R. Jain, L. Mundkur, H.R. Rajalakshmi, P. Lad, P. Neupane, A randomized study to determine the sun protection factor of natural pterostilbene from pterocarpus marsupium, Cosmetics, 7 (2020) 16. https://doi.org/10.3390/cosmetics7010016.

[5] D. Bhattacharjee, S. Preethi, A.B. Patil, V. Jain, A comparison of natural and synthetic sunscreen agents: a review, Int. J. Pharm. Sci. Res., 13 (2021) 3494-3505. https://doi.org/10.31838/ijpr/2021.13.01.524.

[6] T. Sharma, V. Tyagi, M. Bansal, Determination of sun protection factor of vegetable and fruit extracts using UV–Visible spectroscopy: A green approach, Sustain. Chem. Pharm., 18 (2020) 100347. https://doi.org/10.1016/j.scp.2020.100347.

[7] A.C. Da Silva, J.P. Paiva, R.R. Diniz, V.M. Dos Anjos, A.B.S. Silva, A.V. Pinto, E.P. Dos Santos, A.C. Leitão, L.M. Cabral, C.R. Rodrigues, Photoprotection assessment of olive (Olea europaea L.) leaves extract standardized to oleuropein: In vitro and in silico approach for improved sunscreens, J. Photochem. Photobiol. B, 193 (2019) 162-171. https://doi.org/10.1016/j.jphotobiol.2019.03.003.

[8] M. Radice, S. Manfredini, P. Ziosi, V. Dissette, P. Buso, A. Fallacara, S. Vertuani, Herbal extracts, lichens and biomolecules as natural photo-protection alternatives to synthetic UV filters. A systematic review, Fitoterapia, 114 (2016) 144-162. https://doi.org/10.1016/j.fitote.2016.09.003.

[9] B. Prasanth, A. Soman, J. Jobin, P.S. Narayanan, A.P. John, Plants and phytoconstituents having sunscreen activity, World J. Curr. Med. Pharm. Res., 2 (2020) 14-20. https://doi.org/10.37022/WJCMPR.2020.02019.

[10] F. Merlin, W. Ratnasooriya, R. Pathirana, In vitro investigation of sunscreen activity and evaluation of phytochemical profile of methanolic leaf extract of Rauvolfia tetraphylla, J. Pharmacogn. Phytochem., 9 (2020) 2063-2067. https://www.phytojournal.com/

[11] A.D. Permana, R.N. Utami, A.J. Courtenay, M.A. Manggau, R.F. Donnelly, L. Rahman, Phytosomal nanocarriers as platforms for improved delivery of natural antioxidant and photoprotective compounds in propolis: An approach for enhanced both dissolution behaviour in biorelevant media and skin retention profiles, J. Photochem. Photobiol., 205 (2020) 111846. https://doi.org/10.1016/j.jphotobiol.2020.111846.

[12] M. Hupel, N. Poupart, E.A. Gall, Development of a new in vitro method to evaluate the photoprotective sunscreen activity of plant extracts against high UV-B radiation, Talanta, 86 (2011) 362-371. https://doi.org/10.1016/j.talanta.2011.09.029.

[13] M.H. Boskabady, M.N. Shafei, Z. Saberi, S. Amini, Pharmacological effects of Rosa damascena, Iran. J. Basic Med. Sci., 14 (2011) 295. https://ijbms.mums.ac.ir/

[14] M. Mahboubi, Rosa damascena as holy ancient herb with novel applications, J. Tradit.

Complement. Med., 6 (2016) 10-16. https://doi.org/10.1016/j.jtcme.2015.09.005.

[15] N. Kumar, B. Singh, V.K. Kaul, Flavonoids from Rosa damascena Mill, Nat. Prod. Commun., 1 (2006) 1934578X0600100805. https://doi.org/10.1177/1934578X0600100805.

[16] N.G. Baydar, H. Baydar, Phenolic compounds, antiradical activity and antioxidant capacity of oil-bearing rose (Rosa damascena Mill.) extracts, Ind. Crops Prod., 41 (2013) 375-380. https://doi.org/10.1016/j.indcrop.2012.04.045.

[17] N. Yassa, F. Masoomi, R. Rohani, Chemical composition and antioxidant activity of the extract and essential oil of Rosa damascena from Iran, Population of Guilan, DARU J. Pharm. Sci.,, 17 (2009) 175-180. http://daru.tums.ac.ir/index.php/daru/article/view/541

[18] V. Hajhashemi, A. Ghannadi, M. Hajiloo, Analgesic and anti-inflammatory effects of Rosa damascena hydroalcoholic extract and its essential oil in animal models, Iran. J. Pharm. Res., 9 (2010) 163. https://pubmed.ncbi.nlm.nih.gov/24363723/

[19] G. Latifi, A. Ghannadi, M. Minaiyan, Anti-inflammatory effect of volatile oil and hydroalcoholic extract of Rosa damascena Mill. on acetic acid-induced colitis in rats, Res. Pharm. Sci., 10 (2015) 514. https://www.rpsjournal.net/

[20] F. Fatemi, A. Golbodagh, R. Hojihosseini, A. Dadkhah, K. Akbarzadeh, S. Dini, M.R.M. Malayeri, Anti-inflammatory effects of deuterium-depleted water plus rosa damascena mill. Essential oil via cyclooxygenase-2 pathway in rats, Turk. J. Pharm. Sci., 17 (2020) 99. https://doi.org/10.4274/tjps.galenos.2018.24381.

[21] M. Behbahani, Evaluation of in vitro anticancer activity of Ocimum basilicum, Alhagi maurorum, Calendula officinalis and their parasite Cuscuta campestris, PloS One, 9 (2014) e116049. https://doi.org/10.1371/journal.pone.0116049.

[22] E.K. Selvi, H. Turumtay, A. Demir, E.A. Turumtay, Phytochemical profiling and evaluation of the hepatoprotective effect of Cuscuta campestris by high-performance liquid chromatography with diode array detection, Anal. Lett., 51 (2018) 1464-1478. https://doi.org/10.1080/00032719.2017.1382502.

[23] M. Moradzadeh, A. Hosseini, H. Rakhshandeh, A. Aghaei, H.R. Sadeghnia, Cuscuta campestris induces apoptosis by increasing reactive oxygen species generation in human leukemic cells, Avicenna J. Phytomed., 8 (2018) 237. https://ajp.mums.ac.ir/

[24] W.H. Peng, Y.W. Chen, M.S. Lee, W.T. Chang, J.C. Tsai, Y.C. Lin, M.K. Lin, Hepatoprotective effect of Cuscuta campestris Yunck. whole plant on carbon tetrachloride induced chronic liver injury in mice, Int. J. Mol. Sci., 17 (2016) 2056. https://doi.org/10.3390/ijms17122056.

[25] E. Jafari, A. Bahmanzadegan, G. Ghanbarian, V. Rowshan, Antioxidant activity and total phenolic content from aerial parts of three Cuscuta species, Anal. Chem. Lett., 5 (2015) 377-384. http://dx.doi.org/10.1080/22297928.2016.1143394.

[26] M.M. Akiner, E.K. Selvİ, M. Öztürk, I. Güney, U. Asu, Toxic efficacy of Cuscuta campestris Yunck.(Solanales: Convolvulaceae) and Lupinus albus L.(Fabales: Fabaceae) plant crude extracts against nymphs and adults of Orosanga japonica (Melichar, 1898) (Hemiptera: Ricaniidae) under laboratory conditions, Turk. Entomol. Derg., 45 (2021) 65-75. https://doi.org/10.16970/entoted.743439.

[27] P. Khazaeli, M. Mehrabani, M.R. Heidari, G. Asadikaram, M.L. Najafi, Prevalence of aflatoxin contamination in herbs and spices in different regions of Iran, Iran. J. Public Health, 46 (2017) 1540. https://ijph.tums.ac.ir/index.php/ijph

[28] P. Khazaeli , M. Mehrabani, Screening of sun protective activity of the ethyl acetate extracts of some medicinal plants, Iran. J. Pharm. Res., 1 (2010) 5-9. https://doi.org/10.22037/IJPR.2010.738.

[29] [29] J.D.S. Mansur, M.N.R. Breder, M.C.D.A. Mansur, R.D. Azulay, Determinação do fator de proteção solar por espectrofotometria, An. Bras. Dermatol., 61 (1986) 121-124. https://www.medscape.com/viewpublication/2466_33

[30] M. Mostafavi, I. Sharifi, S. Farajzadeh, P. Khazaeli, H. Sharifi, E. Pourseyedi, S. Kakooei, M. Bamorovat, A. Keyhani, M.H. Parizi, Niosomal formulation of amphotericin B alone and in combination with glucantime: In vitro and in vivo leishmanicidal effects, Biomed. Pharmacother., 116 (2019) 108942. https://doi.org/10.1016/j.biopha.2019.108942.

[31] B. Behnam, M. Rezazadehkermani, S. Ahmadzadeh, A. Mokhtarzadeh, S.N. Nematollahi-Mahani, A. Pardakhty, Microniosomes for concurrent doxorubicin and iron oxide nanoparticles loading; preparation, characterization and cytotoxicity studies, Artif. Cells Nanomed. Biotechnol., 46 (2018) 118-125. https://doi.org/10.1080/21691401.2017.1296850.

[32] E. Mohammadi, M. Zeinali, M. Mohammadi-Sardoo, M. Iranpour, B. Behnam, A. Mandegary, The effects of functionalization of carbon nanotubes on toxicological parameters in mice, Hum. Exp. Toxicol., 39 (2020) 1147-1167. https://doi.org/10.1177/0960327119899988.

[33] R. Mohammadinejad, A. Dehshahri, H. Sassan, B. Behnam, M. Ashrafizadeh, A. Samareh Gholami, A. Pardakhty, A. Mandegary, Preparation of carbon dot as a potential CRISPR/Cas9 plasmid delivery system for lung cancer cells, Minerva Biotecnol., 32 (2020) 106-113. https://doi.org/10.23736/S1120-4826.20.02618-X.

[34] M. Moballegh-Nasery, A. Mandegary, T. Eslaminejad, M. Zeinali, A. Pardakhti, B. Behnam, M. Mohammadi, Cytotoxicity evaluation of curcumin-loaded affibody-decorated liposomes against breast cancerous cell lines, J. Liposome Res., 31 (2021) 189-194. https://doi.org/10.1080/08982104.2020.1755981.

[35] D. Nathalie, G. Yannick, B. Caroline, D. Sandrine, F. Claude, C. Corinne, F. Pierre-Jacques, Assessment of the phototoxic hazard of some essential oils using modified 3T3 neutral red uptake assay, Toxicol. In Vitro, 20 (2006) 480-489. https://doi.org/10.1016/j.tiv.2005.08.018.

[36] A. Da Silva Fernandes, L.B. Brito, G.A.R. Oliveira, E.R.A. Ferraz, H. Evangelista, J.L. Mazzei, I. Felzenszwalb, Evaluation of the acute toxicity, phototoxicity and embryotoxicity of a residual aqueous fraction from extract of the Antarctic moss Sanionia uncinata, BMC Pharmacol. Toxicol., 20 (2019) 1-10. https://doi.org/10.1186/s40360-019-0353-3.

[37] Z. Hashemi, M.A. Ebrahimzadeh, M. Khalili, Sun protection factor, total phenol, flavonoid contents and antioxidant activity of medicinal plants from Iran, Trop. J. Pharm. Res., 18 (2019) 1443-1448. https://doi.org/10.4314/tjpr.v18i7.11.

[38] M.A. Ebrahimzadeh, R. Enayatifard, M. Khalili, M. Ghaffarloo, M. Saeedi, J.Y. Charati, Correlation between sun protection factor and antioxidant activity, phenol and flavonoid contents of some medicinal plants, Iran. J. Pharm. Res., 13 (2014) 1041. https://brieflands.com/journals/iranian-journal-of-pharmaceutical-research/

[39] L.F. Amaral, P. Moriel, M.A. Foglio, P.G. Mazzola, Evaluation of the cytotoxicity and phototoxicity of Caryocar brasiliense supercritical carbon dioxide extract, BMC Complement. Altern. Med., 14 (2014) 1-6. https://doi.org/10.1186/1472-6882-14-450.

[40] A.R. Svobodová, B. Zálešák, D. Biedermann, J. Ulrichová, J. Vostálová, Phototoxic potential of silymarin and its bioactive components, J. Photochem. Photobiol. B, 156 (2016) 61-68. https://doi.org/10.1016/j.jphotobiol.2016.01.011.