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Photoprotective activity of *Buddleja scordioides*

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Abstract

The purpose of this study was to determine the photoprotective properties of the methanolic extract of *Buddleja scordioides*, as well as verbascoside and linarin which were isolated from this extract, and linarin acetate prepared in the laboratory. The photoprotective effect of substances against UV-B induced cellular death was evaluated by challenge experiments using *Escherichia coli*. Verbascoside and linarin acetate showed the highest protection. The sun protection factor (SPF) of the methanolic extract, linarin, linarin acetate, and verbascoside was evaluated by guinea pig bioassays. Verbascoside showed the largest SPF measurement.

Keywords: *Buddleja scordioides*; Linarin; Verbascoside; Photoprotective activity; Sun protection factor

1. Introduction

Sun radiation constantly impacts the earth's surface with approximately 50% of visible light (400–800 nm), 40% of infrared radiation (IR) (1300–1700 nm), and 10% of

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ultraviolet radiation (UV) (10–400 nm). UV is divided conventionally in UV-A (320–400 nm), UV-B (290–320 nm), UV-C (100–290 nm), and vacuo UV (10–100 nm) [1].

In the last decades UV-B radiation reached the planet with higher intensity as a consequence of the ozone layer depletion in the upper atmosphere [2]. It has been reported that adverse effects by UV-B radiation on the human skin include erythema (or sunburn), accelerated skin ageing, and induction of skin cancer [3].

Sunscreens are chemicals that provide protection against the adverse effects of solar and, in particular, UV radiation [4]. Studies in animals have shown that a variety of sunscreens can reduce the carcinogenic and immunosuppressive effects of the sunlight [5].

Natural substances extracted from plants have been recently considered as potential sunscreen resources because of their ultraviolet ray absorption on the UV region [6] and of their antioxidant power [7]. Green tea polyphenols, *Aloe barbadencis* extract, and aromatic compounds isolated from lichens are examples of natural substances evaluated for their sunscreen properties [4,8,9].

Buddleja scordioides HBK (Buddlejaceae) is a shrub which grows in the Chihuahuan desert [10]. Decoctions of this plant are used orally or topically for treating several illnesses such as diarrhea, headache, and hurts. [11]. In the community of Doctor Arroyo, town in Nuevo León, México, *B. scordioides* is known as "escobilla" and rural laborers use the water–alcohol extracts from aerial parts of this shrub as a sunscreen (personal communication).

In the present study, we isolated two active compounds from *B. scordioides* and evaluated their photoprotective activity using bacteria and guinea pig as experimental biological systems.

2. Experimental

2.1. Plant material

The aerial parts of *B. scordioides* were collected in vicinity of Doctor Arroyo, Nuevo León, México, in November 1998. A voucher specimen (Izta 26140) was deposited in Izta Herbarium, FES-Iztacala-National University of Mexico.

2.2. Extraction procedure

Dried and ground aerial parts of *B. scordioides* (2000 g) were extracted with hexane, EtOAc, and MeOH, successively. MeOH extract was concentrated in vacuo giving a residue (15.15%). 3 g of the residue was employed for photoprotective experiments and 300 g was Si-gel CC eluting with CH_2Cl_2–MeOH 19:1 and increasing concentrations of MeOH. Fractions CH_2Cl_2–MeOH (9:1 and 8:2) gave linarin (3 g) and verbascoside (11 g), respectively.

Linarin (**1**). Identified by comparison with reported physical and spectroscopic data [12,13].

Verbascoside (**2**). Identified by comparison with reported physical and spectroscopic data [14].

2.3. Linarin peracetate

Linarin (100 mg) treated with Ac_2O–pyridine at 60 °C for 1 h and kept overnight at 25 °C gave linarin peracetate (**1a**), 68 mg. Identified by comparison with an authentic sample.

2.4. Animals

H-D guinea pigs of both sexes weighing 300–350 g were used. They were housed in standard environmental conditions, fed with standard rodent diet with water ad libitum.

2.5. Photoprotective activity

2.5.1. Protective effect against UV-B induced cell death

A strain of *Escherichia coli* (ATCC 25922) was grown in heart and brain infusion broth (Bioxon-112) until the culture reached a concentration of 10^7–10^8 cells/ml (O.D. 0.3 read at 550 nm). The bacteria were centrifuged 10 min at 6000 rev/min, suspended in Ringer PBS (pH 7.0), and transferred into quartz cuvettes (Pye Unicam B538751 A, thickness 1 mm, capacity 4 ml). Each photoprotective substance was dissolved in an appropriated solvent (2 mg/ml) and put in a quartz cuvette. Cuvette containing bacteria was placed behind the cuvette containing the photoprotective substance, forming one experimental unit. The experimental units were irradiated with an UV-B lamp (312 nm, Spectroline EB-280C) [15]. Irradiation dose was 0.60 J/cm^2. The survivor bacteria number was detected in accordance with the dilution method [16] at different time periods. The substances employed were *B. scordioides* MeOH extract, verbascoside in MeOH, linarin in DMSO, and linarin peracetate in $CHCl_3$. The positive control was escalol (ethylhexyl-methoxycinnamate, ISP VAN DIK) and the negative controls were the solvents used for dissolving the photoprotective substances.

2.5.2. Sun protection factor (SPF) measurements

Dorsal skin of guinea pigs was shaved with electric clippers (Oster Mod. 274-01) and then depilated with lotion hair remover (Velvinette-Wella). The skin was rinsed under warm tap water and dried with a towel. After 16 h, dorsal skin was treated with 2 mg/cm^2 of photoprotective substances or with vehicle (setting gel-Stil Net) or was left untreated. Animals (5 for each group) were then wrapped with 7.5 cm wide tape (Tuk M.R. #4) containing six exposure windows (three windows on each side of the spinal line) of 2.0 cm^2. After 15 min animals were placed under a bank of 5 UV-B lamps (312 nm, Spectroline EB-280C) positioned 15 cm above their backs. The irradiation at this distance was of 0.60 J/cm^2 measured with a Spectroline model DM-300HA research radiometer. Irradiation times of the six exposure windows on each animal were set to bracket the suspected SPF of the substance being tested. Exposure windows were covered with opaque tape at the end of each time point. At the completion of irradiations, tape was removed from animals. Erythema was scored (0–3 grading scale, with half-grade increments) 24 h later, using non-exposed adjacent skin on each animal as a non-UV control (score = 0). A grade of 1.0

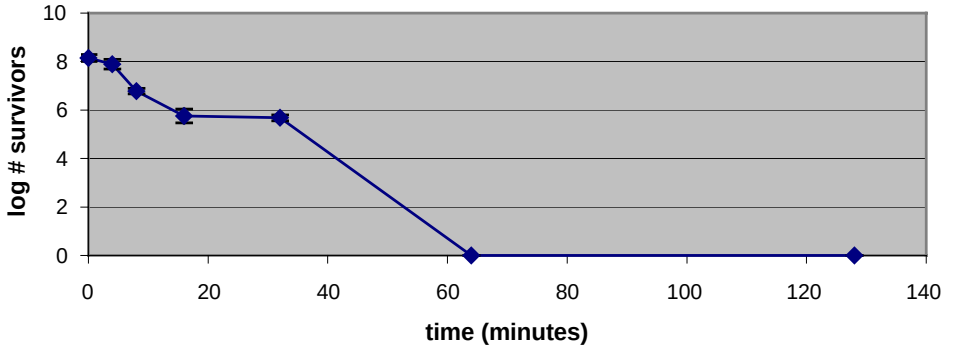

Fig. 1. Photoprotection of methanolic extract of *B. scordioides* on *E. coli* against UV-B (312 nm) irradation ($K=0.06$, $R^2=0.97$).

(detectable redness over the entire exposure area) was considered to be 1 Minimal Erythema Detectable (1 MED). The SPF was then calculated from the ratio: (UV dose to achieve 1 MED with photoprotective substance)/(UV dose to achieve 1 MED without photoprotective substance) [17]. The erythema responses to UV exposure of vehicle-treated and untreated skin were identical (SPF = 1). Twenty-four hours after SPF determination, biopsies were taken from irradiated zones at the maximum irradiation time without MED of each treatment, then the animals were killed. Samples of dorsal skin tissue were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 6 to 8 μm. Sections were stained with hematoxylin and eosin dyes (H&E stain).

2.6. Statistical analysis

In the study of photoprotective effect with bacteria, the mortality rate (K) and determination coefficient (R^2) were obtained using the mortality Gomperts function. In

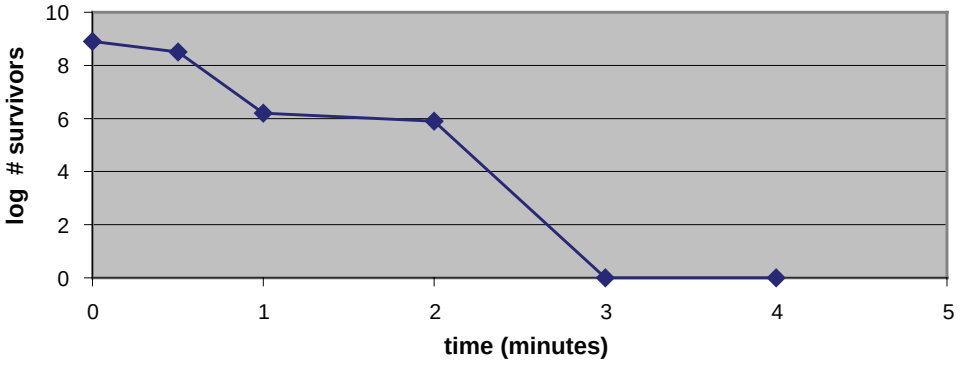

Fig. 2. Cellular death of *E. coli* exposed to UV-B irradation (312 nm) without protection ($K=1.44$, $R^2=0.95$).

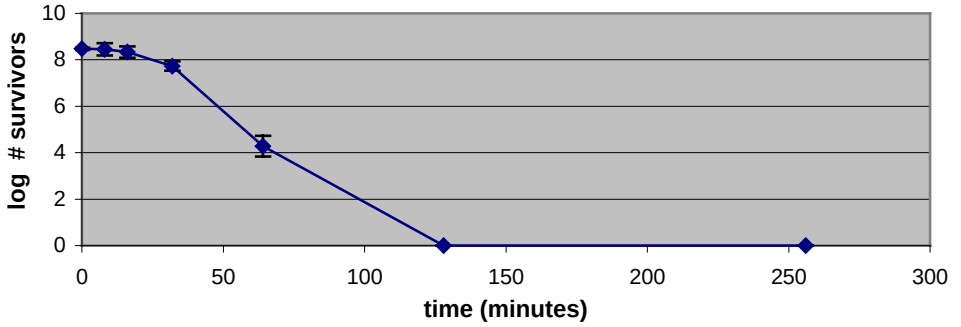

Fig. 3. Photoprotection of escalol on *E. coli* against UV-B irradation (312 nm) ($K=0.060$, $R^2=0.99$).

the SPF determination, the statistical significance among treatment groups at specific time points was determined using the non-parametric Friedman test. The significant difference between irradiation times was determined by the Wilcoxon test.

3. Results

3.1. Protective effect against UV-B induced cell death

The protective effect against UV-B induced cell death were evaluated using *E. coli* (ATCC 25922) as a cell model. The results showed that the methanolic extract of *B. scordioides* possesses a pronounced photoprotective activity compared to the negative control even if resulted less active than escalol as a positive control. In fact, the bacteria population ($\cong 10^8$ cells/ml), protected with methanolic extract of *B. scordioides*, reached cell death at 37–65 min interval (Fig. 1); negative and positive control, in turn (Figs. 2 and 3), reached cell death at 180 s and 65–130 min intervals, respectively. Linarin (**1**) and verbascoside (**2**) were isolated from the methanolic extract of *B. scordioides*, and linarin peracetate (**1a**) was prepared in our laboratory. These compounds were tested for their

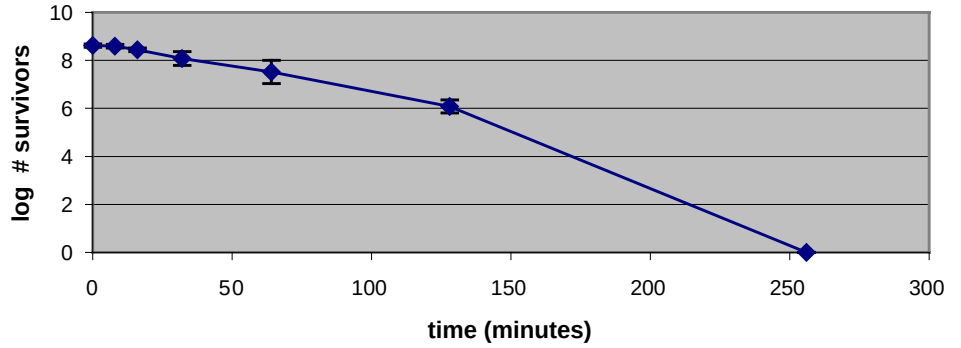

Fig. 4. Photoprotection of linarin acetate against UV-B (312 nm) irradation on *E. coli* ($K=0.019$, $R^2=0.99$).

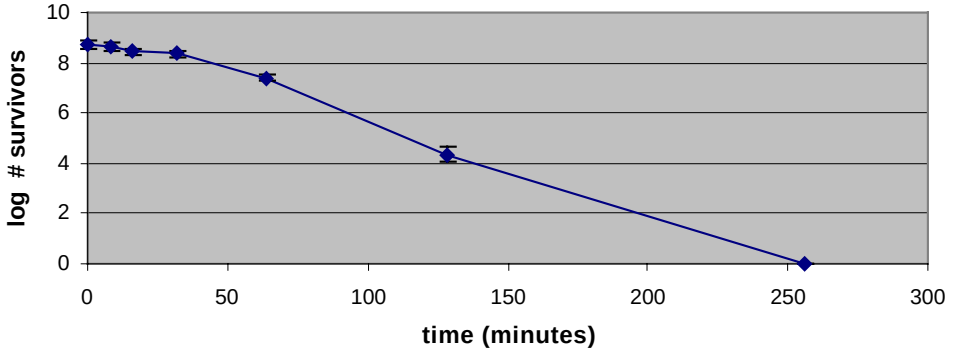

Fig. 5. Photoprotection of verbascoside against UV-B (312 nm) irradation on *E. coli* ($K=0.025$, $R^2=0.99$).

protective effect against UV-B induced cell death. Figs. 4–6 show the photoprotective effect. Linarin peracetate and verbascoside protected bacteria more efficiently than positive control and methanolic extract. Bacterial population protected by these compounds reached cell death until 125–250 min interval, while bacteria protected with linarin reached cell death until 40–80 min interval.

Linarin (**1**), verbascoside (**2**) and linarin peracetate (**1a**) are reported in Fig. 7.

3.2. Sun protection factor (SPF)

The SPF values of the tested substances were determined on guinea pigs (Table 1). Negative control (guinea pigs protected only with vehicle) showed perceptible erythema at 20 ± 2 min; this time was considered as 1 SPF. Methanolic extract had the smallest SPF, probably due to low concentration of the photoprotective compounds in the extract. Verbascoside was the substance with the highest photoprotective activity, its SPF was of 24 ± 0.7 retarding the erythema appearance until 440 ± 14 min. Linarin acetate protected

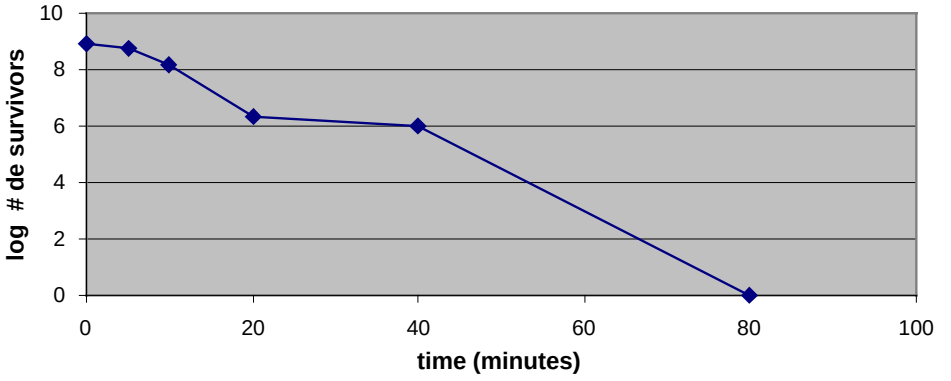

Fig. 6. Photoprotection of linarin against UV-B (312 nm) irradation on *E. coli* ($K=0.051$, $R^2=0.96$).

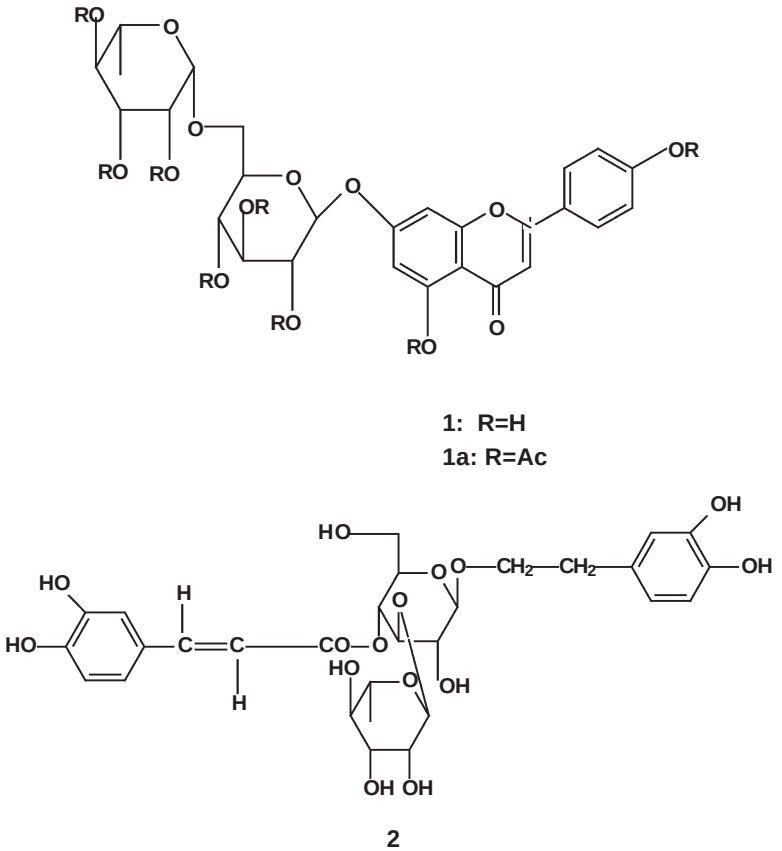

Fig. 7. Linarin (**1**), linarin peracetate (**1a**), verbascoside (**2**).

skin in similar manner than positive control (escalol). The SPF obtained for linarin was of 9 ± 0.3.

According to Friedman test, there were significant differences in each treatment. Wilcoxon test showed significant differences between negative control and experimental

Table 1
Sun protection factor (SPF) in guinea pigs

Tested (2 mg/cm^2)	SPF	Exposition time without erythema (min)
Control *B. scordioides*	1	20 ± 2
MeOH extract	3 ± 0.09	40 ± 1.8
Verbascoside	24 ± 0.7	440 ± 14
Linarin	9 ± 0.3	160 ± 6
Linarin acetate	5 ± 0.2	80 ± 4
Escalol	5 ± 0.1	80 ± 2

Table 2
Statistical significance according to Friedman and Wilcoxon tests

Friedman test:				
MeOH extract of *B. scordioides*	Verbascoside	Linarin	Linarin acetate	Escalol
$\chi^2=10.78$, $p<0.05$	$\chi^2=37.5$, $p<0.005$	$\chi^2=28.27$, $p<0.005$	$\chi^2=28.27$, $p<0.005$	$\chi^2=28.27$, $p<0.005$

Wilcoxon test:									
MeOH extract of *B. scordioides*		Verbascoside		Linarin		Linarin acetate		Escalol	
MED	*P*-value	MED	*P*-value	MED	*P*-value	MED	*P*-value	MED	*P*-value
1-2	0.046	1-2	0.025	1-2	0.025	1-2	0.025	1-2	0.025
1-3	0.083 n.s.	1-4	0.025	1-4	0.046	1-4	0.046	1-4	0.025
1-8	0.083 n.s.	1-8	0.025	1-8	0.025	1-5	0.317 n.s.	1-5	0.083 n.s.
1-16	0.157 n.s.	1-16	0.025	1-9	0.157 n.s.	1-8	0.500 n.s.	1-8	0.083 n.s.
1-20	0.157 n.s.	1-22	0.025	1-16	0.157 n.s.	1-16	0.500 n.s.		
		1-24	0.083 n.s.	1-22	0.317 n.s.				
		1-32	0.570 n.s.	1-28	0.059 n.s.				

n.s. = no significant difference.

substances during the time of effective photoprotection (Table 2), consequently the SPF values obtained from UV-B absorbing substances are reliable.

4. Discussion

The results showed that methanolic extract of *B. scordioides* possesses a photoprotective activity as showed on the effect against UV-B induced cell death. Also, linarin and verbascoside isolated from the extract and linarin peracetate prepared in our laboratory protected bacteria efficiently.

In the in vivo test methanolic extract showed the smallest sun protection factor (SPF) probably due to low concentration of the photoprotective compounds in the extract. In contrast with photoprotection in bacteria experiments, linarin had larger SPF than linarin peracetate; probably linarin peracetate is absorbed across the skin due to its high lipofilicity.

In all cases methanolic extract, isolated compounds, and positive control protected the skin tissue from irradiation burn because there was no evidence of histological changes, while negative control exhibited cellular (vacuolization) and vascular inflammatory alterations (edema), as well as *stratum corneum* detachment (data not shown).

Verbascoside was the best photoprotector compound because the protection against cellular death and SPF values were greater than those obtained from linarin, linarin peracetate, and escalol. In accordance with Elmets and Young [4], the sunscreens should have two activities: first, furnish a SPF higher than 15 and, second, have antioxidant properties. Verbascoside could be a good sunscreen because its SPF is higher than 15, and it has antioxidant and wound healing properties [1].

It is probable that phenolics compounds such as verbascoside and linarin provide chemical defense against UV radiation in *B. scordioides*, because similar substances in some other plant species fulfill such functions [18].

In summary, our study clearly correlates the popular use of *B. scordioides* with its photoprotective effect. The methanolic extract of *B. scordioides* has photoprotective activity; this may be explained by the presence of absorbing UV-B compounds in the extract such as verbascoside and linarin. Results show that verbascoside has photoprotection quality better than the other tested compounds. The advantage of verbascoside could be represented by the good extinction coefficient and by their antioxidant properties.

Acknowledgements

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