Nutrifarm

bem-viver sem medida

Portulaca

1. Propriedades

Portulaca oleracea é uma planta que pertence a família Portulacea e e está distribuída em muitas partes do mundo, como Himalaia, Rússia e Grécia.

O extrato de portulaca é uma rica fonte de diversos nutrientes, tais como vitaminas, minerais, glutationa ácido glutâmico, ácido aspártico e ácido graxo (particulamente ômega-3).

Devido a seus efeitos notáveis no tratamento de desordem cutâneas, foi amplamente utilizada na medicina tradicional oriental através de compressas ou banhos para aliviar inchaços, coceiras, furúnculos, eczema e também como desintoxicante de venenos de insetos. Está listada na Organização Mundial da Saúde (como uma das plantas terâpeuticas mais utilizadas, possui diversas propriedades farmacológicas como analgésico, antibacteriano, relaxante muscular esquelético, cicatrização de feridas, anti-inflamatório e antioxidante, sendo considerado também um excelente protetor contra raios UV e calmante da pele.

Um estudo publicado pelo "J. Ethnopharmacol, 2000 demonstrou que o uso tópico de Portulaca apresentou uma significativa ação anti inflamatória e analgésica comparada com drogas sintéticas como o diclofenaco de sódio.*

Portulaca Extract®

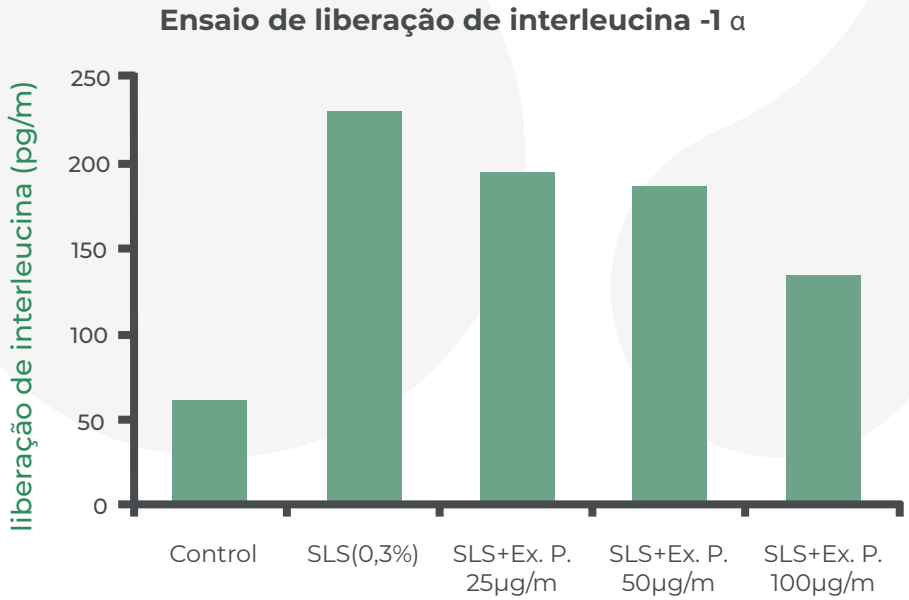
Anti-Inflamatório, Anti-Irritante e Anti-Fototóxico

*Estudo completo no final da literatura

2. Teste *EX-VIVO*

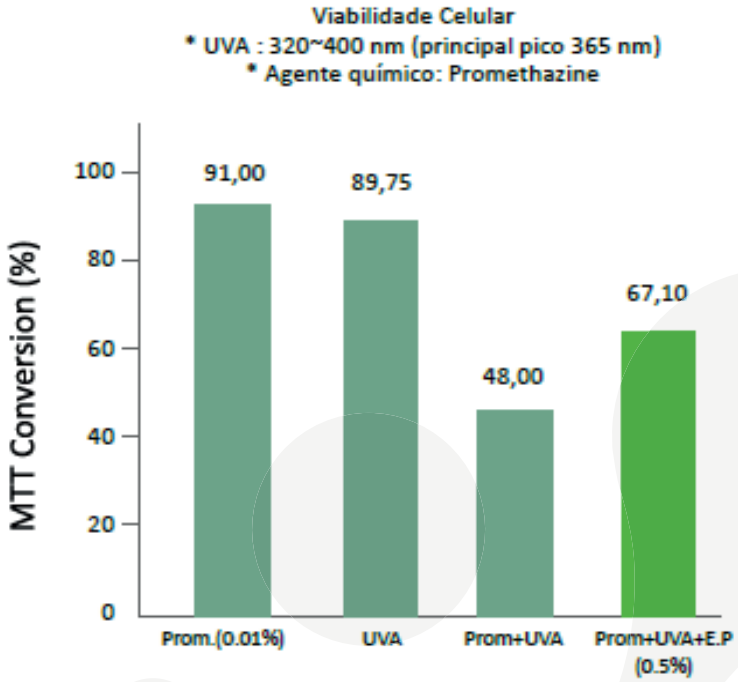
De acordo com o gráfico abaixo, o estudo *ex vivo* demonstrou a **ação anti-inflamatória** do extrato de portulaca na pele artificial com inflamação induzida por Lauril Sulfato de sódio.

O extrato de portulaca nas dosagens 2 5 5 e 10 reduziram a liberação do mediador pró inflamatório Interleucina 1 alfa (um dos mais importantes marcadores de indução da resposta inflamatória)



3. Teste *IN VITRO*

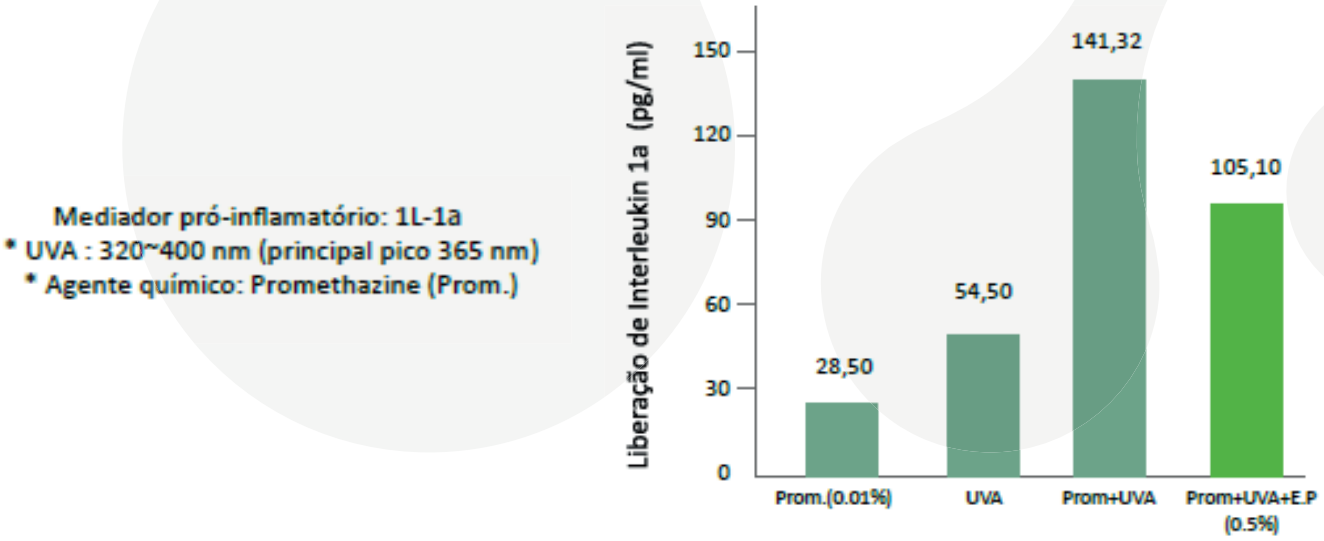
3.1 Fototoxicidade - Varibialidade celular



O teste apresenta um aumento significativo na viabilidade celular quando comparado à amostra sem Extrato de Portulaca®

3.2 Fototoxicidade - Mediador pró - inflamatório

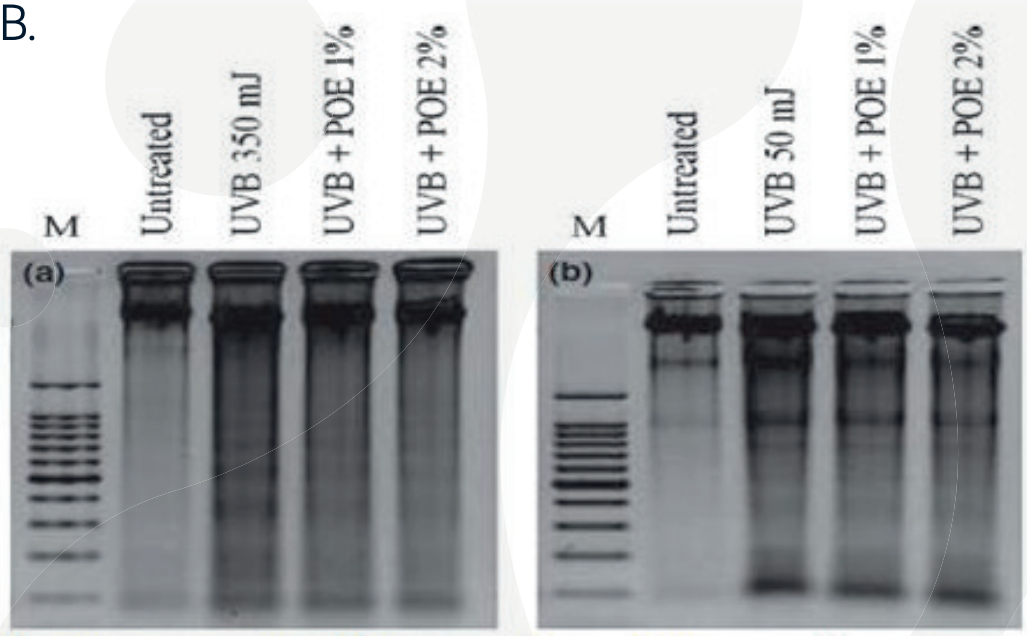
Neste gráfico observa se redução na liberação de mediadores pró inflamatórios na amostra contendo Extrato de Portulaca®



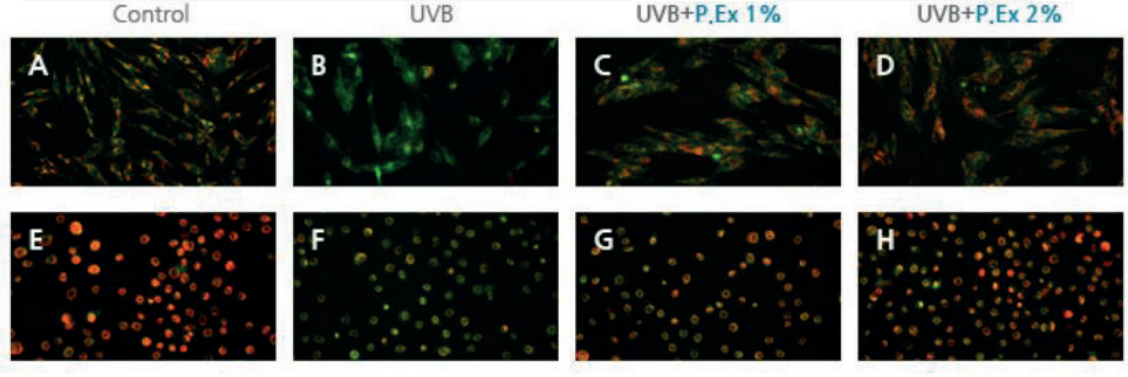
3.3. Proteção celular contra UV**

Neste estudo, foi investigado o papel o extrato de Portulaca na apoptose, induzida por UVB, de fibroblatos e queratinócitos através da fragmentação do DNA.

Os resultados do estudo mostram que o extrato de portulaca protege os fibroblastos e os queratinócitos de danos induzidos por UVB Em particular, o extrato de portulaca reduz efetivamente a morte celular e a apoptose, após a irradiação com UVB. Assim, sugere se que o extrato de portulaca é um ativo que pode ser utilizado como ingrediente cosmético eficaz para prevenir a indução de danos causados pelo UVB.



Detecção de Fragmentação de DNA. O DNA foi extraído de células tratadas ou não tratadas com extrato de Portulaca oleracea com ou sem Irradiação com UVB. O DNA foi submetido a eletroforese em gel de agarose a 1,5%. (a) está ligado fibroblasto e (b) está em queratinócitos. (M: marcador DNA 100 bp)



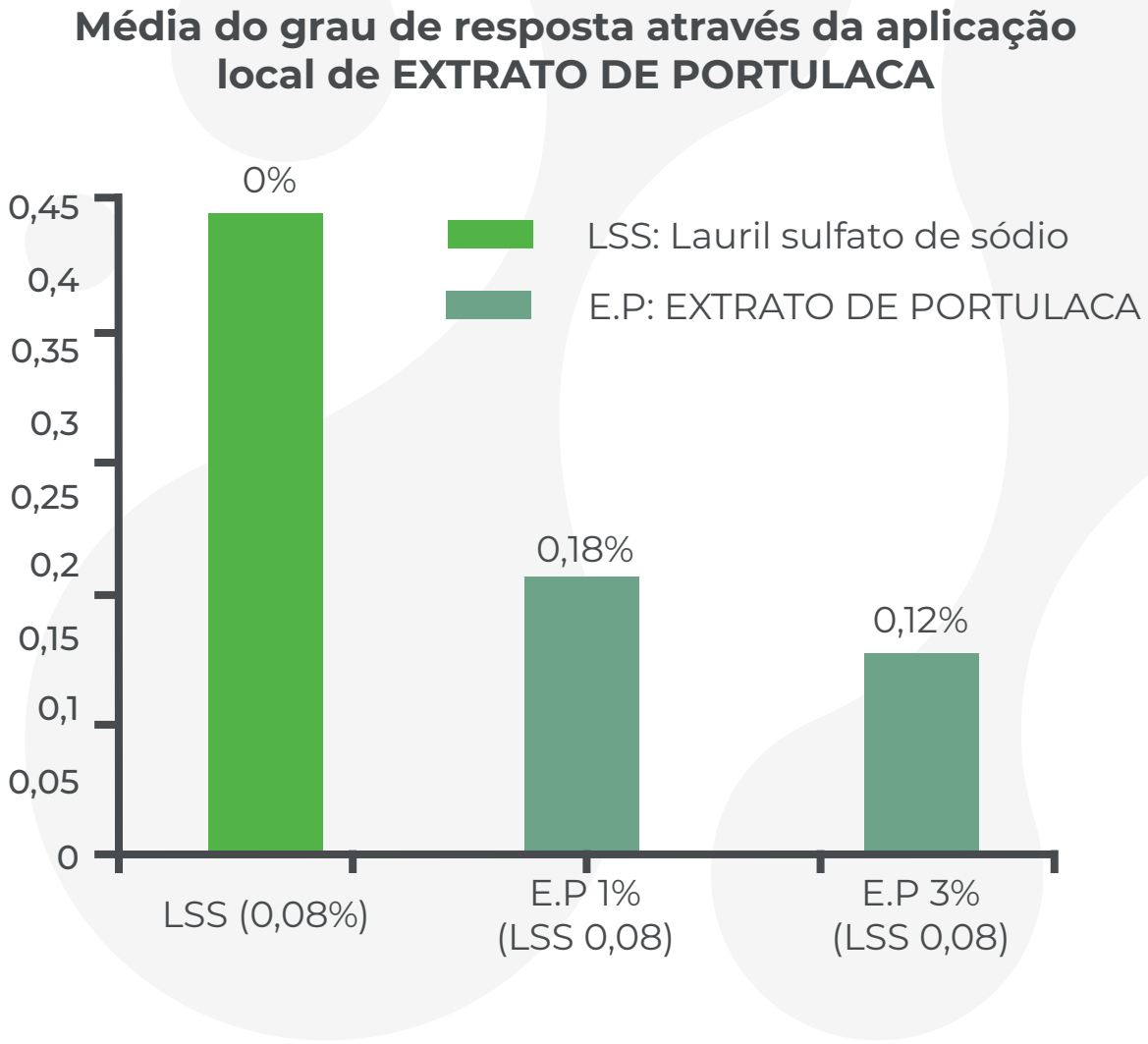
A~D : Fibroblast against UVB 350 mJ/cm² / E~F : Keratinocyte ageinst UVB 50 Mj/cm²

**Estudo completo no final da literatura

4. Teste *IN VITRO*

4.1. Ação anti irritante

O teste de contato é o método mais eficiente para confirmar o diagnóstico etiológico alérgico de contato. A presença de teste positivo a certa substância, possibilita identificar os materiais que em contato com a pele do individuo pode desencadear um quadro de irritação
O teste de contato humano foi realizado com 50 voluntários durante 24 horas. O **agente irritante** utilizado foi o **Lauril Sulfato de Sódio (SLL) à 0,08%.**



De acordo com os resultados do gráfico acima, o extrato de Portulaca® apresentou uma excelente resposta anti-irritante.

5. Sugestão de uso

Extrato de Portulaca® é um ingrediente natural para produtos cosméticos e farmacêuticos, com ação anti-inflamatória, anti-irritante e antifototoxica.

Ideal para a produção de produtos infantis, peles oleosas e acneicas peles sensíveis, proteção solar, tratamento de caspa e tratamento de dermatite atópica.

Concentração usual 0,5% - 10% de extrato de portulaca (hidrossolúvel).

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The analgesic and anti-inflammatory effects of *Portulaca oleracea* L. subsp. *sativa* (Haw.) Celak

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Abstract

Many ethnic groups have used different species of *Portulaca oleracea* L., a member of the Family Portulacaceae, as vegetable and also herbal medicine against several diseases for many centuries. A review of the records in both folkloric and scientific literature indicates that *Portulaca* has many medicinal uses. After our previous preliminary screening of three species of the family for analgesic and anti-inflammatory properties, *Portulaca oleracea* L. subsp. *sativa* (Haw.) Celak. (a cultivar) was chosen for further work due to its abundant availability from reliable sources. The 10% ethanolic extract of the aerial parts (dried leaves and stem) showed significant anti-inflammatory and analgesic after intraperitoneal and topical but not oral administration when compared with the synthetic drug, diclofenac sodium as the active control. Results indicate this cultivar species of *Portulaca* also possesses some of the claimed traditional uses of the wild species in the relief of pain and inflammation.

Keywords: Analgesic; Anti-inflammatory; Pharmacological actions; *Portulaca oleracea* L.; *Portulaca oleracea* L. subsp. *sativa* (Haw.) Celak

1. Introduction

Portulaca oleracea L. subsp. *sativa* (Haw.) Celak. (Portulacaceae) has a cosmopolitan distribution, but in the Mediterranean region it is distributed in the arid lands, Sah-Sind, Sahil region, Somali-Masai, and Ir-Tur. In Arabia (Miller

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and Cope, 1996) it is found in Saudi Arabia (Mandaville, 1990), UAE (Western, 1986), and Yemen (Al-Gifri, 1992). *P. oleracea* subsp. *sativa* is the variety cultivated as vegetable throughout the regions. It is perhaps best regarded as a selected form of subsp. *oleracea* (Danin and Baker, 1978; Thulin, 1993).

1.1. Folkloric uses of Portulaca

In the United Arab Emirates (UAE) and Oman, the cultivated variety, *P. oleracea* L. subsp. *sativa* (Haw.) Celak. is available in many vegetable shops being used as salad (Miller and Morris, 1988). It is used as febrifuge (El-Ghonemy, 1993). In some other Arabian countries, it is used as anti-scorbutic, antiseptic, antispasmodic, diuretic, vermifuge, refrigerant, and as a vulnerary herb against sore nipples, ulcers of the mouth and urinary disorders (Ghazanfar, 1994).

In early Muslim source (Miller and Morris, 1988), the plant has been described as being mildly constipating, good for teeth, and soothing to ulcers of the stomach. In the Middle East, the whole plant is regarded as being an aphrodisiac. Extracts of the plant are used to reduce inflammation and as vermifuge; also as a bactericide in bacillary dysentery. A poultice made from the leaves is applied to draw the pus out of infected sores and the seeds are taken as a sedative and to quench the thirst. In the Indian subcontinent, the plant is reputed to reduce ulcers, tumours and inflammation. In Pakistan, it is considered useful as a component of diet in scurvy; useful in haemoptysis, liver obstruction and debility, reducing small tumours and inflammations; good for ulcers, asthma, urinary discharges, diarrhoea, dysentery and piles; effective against tape worms; useful against skin affected by boiling water, warm inflammations (Usmanghani et al., 1997).

1.2. Pharmacology and clinical uses of Portulaca

The key pharmacological studies on *P. oleracea* are summarised in Table 1. Therefore this is a very interesting plant family to investigate scientifically for ascertaining some of the uses described. We have previously showed that the 10% ethanolic extracts of three species of the family, *P. oleracea*, *P. grandiflora* and *P. oleracea* L. subsp. *sativa* (Haw.) Celak., possessed significant analgesic and anti-inflammatory activities when compared with synthetic drugs (Islam et al., 1998; Zakaria et al., 1998). Due to the abundant availability of the cultivar, *P. oleracea* L. subsp. *sativa* (Haw.) Celak., we have chosen to screen in more detail the pharmacological activities of the species. We now report the findings on the analgesic and anti-inflammatory activities of a 10% ethanolic extract of the dried aerial parts (leaves and stems) of the whole mature plants using an experimental model developed in our laboratories. All investigations were conducted with reference to known conventional drugs used clinically.

The present investigation concerned the claimed medicinal uses of some *P. oleracea* species that had been cited in folkloric literature and in scientifically published work (Table 1). While there were quite a few scientific publications on the muscle relaxant actions of the plant extract (mostly aqueous) not many had been reported on the analgesic and anti-inflammatory properties. After our preliminary screening of three species (*P. oleracea*, *P. grandiflora* and *P. oleracea* L. subsp. *sativa* (Haw.) Celak.), on analgesic (Islam et al., 1998) and anti-inflammatory (Zakaria et al., 1998) actions, the present study concentrated on the cultivar species, and *P. oleracea* L. subsp. *sativa* (Haw.) Celak., because of its abundance and reliability for getting a constant supply with guaranteed source of good agricultural practice.

2. Material and methods

2.1. Preparation of crude plant materials

Fresh whole plants of *P. oleracea* L. subsp. *sativa* (Haw.) Celak., were collected from a farm in Al Ain, United Arab Emirates. The plant specimen was authenticated morphologically with external details (herbaceous, erect or decumbent, up to 30 cm high; succulent. Stems cylindrical, up to 30 cm long, 2–3 mm in diameter; greenish; somewhat swollen at nodes; smooth, glabrous apart

Table 1
A summary of literature reported pharmacological studies on *P. oleracea*

Activity	Experimental model	Extract used	Dose	Route	Animal used	Reference
Skeletal muscle relaxant activity	Assessing prolongation of pull-up time in rats	Aqueous extract of aerial parts	200–1000 mg/kg	In vivo p.o. and i.p.	Rat	Parry et al., 1987a
Skeletal muscle relaxant activity	Rat phrenic-nerve-hemidiaphragm preparation	Aqueous, dialysable, ether, methanolic extract of aerial parts	Various doses: $0.82–5.5\times10^{-3}$ g/ml	In vitro studies	Rat	Al-Harbi et al., 1994
Skeletal muscle relaxant activity	Frog's rectus abdominis prepn.	Aqueous, dialysable, ether, methanolic extract of aerial parts	Various doses: $0.6–1.2\times10^{-2}$ g/ml	In vitro studies	Frog	Al-Harbi et al., 1994
Skeletal muscle relaxant activity	Isolated nerve-muscle prepn. (Chick biventer cervics)	Ethanolic extract of aerial parts	100 mg/ml of solution of the extract	In vitro studies	Chicken	Okwausaba et al., 1987
Skeletal muscle relaxant activity	Rat phrenic-nerve-hemidiaphragm preparation	10% ethanolic extract of aerial parts	0.2, 1.6, and 1.8 mg/ml	In vitro studies	Rat	Radhakrishnan et al., 1998
Clinical assessment of skeletal muscle relaxant activity	Examination of the spasticity muscle and exercised muscle tone by electromyograph	Aqueous extract of leaves and stems	0.5–1.0 ml of a solution of 140 mg/ml	Topical	Healthy subject and spastic patient	Parry et al., 1987b
Effect on smooth muscles	Guinea pig fundus; rabbit jejunum and rabbit aorta preparations	Aqueous extract	Various	In vitro	Guinea pig; rabbit	Parry et al., 1988
Effect on blood pressure	Polygraph	Aqueous extract	1.4–5.6 mg/kg	In vivo	Rat	Parry et al., 1988
Neuropharmacology activity of *P. oleracea* var. *sativa*	Activity meter	10% ethanolic extract	200 and 400 mg/kg	In vivo i.p.	Mouse	Radhakrishnan et al., 1998
Anti-convulsant activity	Pentylene-tetrazole induced convulsions	10% ethanolic extract	200 and 400 mg/kg	In vivo i.p.	Mouse	Radhakrishnan et al., 1998

from the leaf axils. Leaves alternate or subopposite; simple; flat, fleshy; shapes variable, obovate, 1–5 cm long, 0.5–2 cm across; obtuse or slightly notched at the apex; tapering at base; sessile or indistinctly petiolate; glabrous, smooth and waxy on the upper surface; margin entire. Stipules small clusters of hairs up to 1 mm long). It was given a voucher number *P. oleracea* 1330, recorded in the ZCHRTM database. The plants were authenticated before they were cleaned by removal of foreign matter. The pharmacognostic and phytochemical profiles were screened and obtained (Kamil et al., 1998) before processing. The leaves were separated from the fresh stems and all were spread on filter paper sheets in thin layers under shade at room temperature (about 25°C) until dried with changing of filter sheets. The drying process took 13 days. The dried aerial parts were then crushed in a mill and stored in a dry place until use. The percentage yield of the dried greyish powdered materials with respect to the fresh plant parts was between 6.5 and 7.5%.

2.2. Preparation of the 10% ethanolic extract

Portions of the dried aerial parts were weighed into a 10-l round-bottomed flask fitted with condenser and a heated mantle. The whole content was refluxed with 10% v/v aqueous ethanol (90 ml distilled water + 10 ml ethanol) for 4 h. The resulting slurry was filtered through Whatman No. 1 filter paper and the residue was again refluxed with fresh solvent as above. The two volumes of the combined filtrate were reduced using a Buchi evaporator under reduced pressure. The concentrated extract was transferred to tared dishes and dried on a water bath and finally in a vacuum oven at 40°C. The solid extract was scraped before complete drying; and then dried to constant weight. This yellowish brown hygroscopic solid extract was kept in a vacuumed dessicator until use.

2.3. Experimental models

2.3.1. Analgesic models

The analgesic activity was evaluated using the hot-plate method and tail-flick response on albino mice and Wistar rats, respectively. The hot-plate reaction time was taken as the time between placing the mouse on the hot plate and the licking of its fore or hind paws. Tail-flick response was measured as the time taken by the rat to withdraw the tail from the radiant heat source. In both methods the reaction time was measured 10 min before, and 2, 4, and 6 h after the i.p. administration of either the 10% ethanolic extract (400 mg/kg as dried weight of herb) or diclofenac sodium (4 mg/kg).

2.3.2. Anti-inflammatory models

Inflammation was induced in the hind paw of Wistar rats by injecting carrageenan s.c. in the subplantar region. Two acute dose levels (200 and 400 mg/kg) of the 10% ethanolic extract of the *Portulaca* aerial parts were administered intraperitoneally or orally, 30 min after carrageenan injection in different groups of animals. The hind paw volume was measured before and after the carrageenan challenge plethysmometrically at 30-min intervals for 6 h and at 24 h. Anti-inflammatory activity of the extract (200 and 400 mg/kg, daily, p.o.) was also assessed using the cotton pellet method (Goldstein et al., 1976) with 6 days of subacute treatment.

2.4. Statistical analysis

Results were calculated as mean + S.E.M. All test parameters were analysed against positive control (action of synthetic drug) and negative control (saline or vehicle) for statistical significance. The significance of the differences between the means of data from different groups of animals was established by the Student's *t*-test. *P*-values less than 0.05 were considered significant.

3. Results

3.1. Analgesic activities

The 10% ethanolic extract of the *P. oleracea* var. *sativa*, aerial part showed significant analgesic properties as assessed by the two analgesic models, tail flick reaction time (Table 2) and the hot

plate reaction time (Table 3). Results indicate that the analgesic effect of the plant extract was comparable with that of the sodium diclofenac solution during the first 6 h after i.p. administration. The 400-mg dose of the extract gave an earlier onset and higher and longer activity of analgesia than the 200-mg dose and the 4 mg diclofenac as active control.

3.2. Anti-inflammatory activities

The 10% ethanolic extract of the *P. oleracea* var. *sativa*, aerial part showed significant anti-inflammatory properties as assessed by the two experimental models, reduction in hind paw volume (Table 4) and reduction of cotton pellet weight. Results were comparable to those obtained from the active control, diclofenac-treated animals.

In the cotton pellet experiment, after intraperitoneal administration of 400 mg/kg of the 10% ethanolic extract of *P. oleracea* var. *sativa* or 4 mg/kg of diclofenac sodium, for 10 days to Wistar rats, the percentage increase in cotton pellet weights in the control ($86.6 \pm 11.8\%$) was significantly ($P < 0.05$) reduced by the 10% ethanolic extract ($40.4 \pm 2.36\%$) and diclofenac, the active control ($52.8 \pm 5.50\%$).

4. Discussion

The 10% ethanolic extract was arbitrarily chosen, after our preliminary screening using aqueous and varying percentage of ethanol in the extracting solvent, in preference to the aqueous or the higher ethanolic content extraction for the follow-

Table 2
Percentage (mean ± S.E.M.) of tail flick reaction time after treatment with the 10% ethanolic extract (200 and 400 mg/kg, i.p.) of *P. oleracea* var. *sativa*, aerial part, compared with diclofenac sodium in water (4 mg/kg, i.p.)

	Time after treatment (h)				
	0	1	2	4	6
Control	100.0 ± 7.56	104.0 ± 9.18	110.2 ± 16.4	112.8 ± 10.8	107.7 ± 12.0
Portulaca extract (200 mg/kg)	100.0 ± 8.89	137.5 ± 13.3	184.4 ± 22.1*,a	187.0 ± 16.1*,a	249.7 ± 28.8*,a
Portulaca extract (400 mg/kg)	100.0 ± 7.57	253.1 ± 53.4*,a	261.2 ± 58.6*,a	201.2 ± 32.2*,a	187.2 ± 32.5*
Diclofenac sodium (4 mg/kg)	100.0 ± 8.50	129.5 ± 8.67	141.1 ± 12.3*	156.7 ± 16.3*,a	157.3 ± 16.5*,a

[a] Significantly different from control value at $P<0.05$.
* Significantly different from zero time (before treatment) at $P<0.05$.

Table 3
Percentage (mean + S.E.M.) of hot plate reaction time after treatment with the 10% ethanolic extract (200 and 400 mg/kg, i.p.) of *P. oleracea* var. *sativa*, aerial part compared with diclofenac sodium (4 mg/kg, i.p.)

	Time after treatment (h)				
	0	1	2	4	6
Control	100.0 ± 3.35	117.5 ± 12.9	102.9 ± 6.72	116.8 ± 17.5	119.6 ± 8.69
P. oleracea var. *sativa* (200 mg/kg)	100.0 ± 4.01	122.4 ± 12.8	111.6 ± 13.9	109.8 ± 13.1	100.8 ± 15.1
P. oleracea var. *sativa* (400 mg/kg)	100.0 ± 1.98	149.4 ± 21.4*	164.2 ± 23.4*,a	163.5 ± 10.7*	172.8 ± 10.7*,a
Diclofenac sodium (4 mg/kg)	100.0 ± 9.19	165.5 ± 35.2	172.5 ± 28.7*	156.7 ± 9.56	155.7 ± 20.5

[a] Significantly different from control value at $P<0.05$.
* Significantly different from zero time (before treatment) at $P<0.05$.

Table 4
Percentage of increase of hind paw volume after treatment with *P. oleracea* var. *sativa*, aerial part, 10% ethanolic extract (200 and 400 mg/kg, i.p.) compared with diclofenac sodium (4 mg/kg, i.p.)

Time (min)	Control	*P. oleracea* var. *sativa* (200 mg/kg)	*P. oleracea* var. *sativa* (400 mg/kg)	Diclofenac sodium (4 mg/kg)
0	0.00	0.00	0.00	0.00
30	21.1 ± 2.52*	14.0 ± 2.18*	26.2 ± 3.60*	8.7 ± 2.67*,a
60	51.0 ± 2.03*	42.9 ± 5.25*	47.8 ± 4.71*	10.5 ± 2.32*,a
90	77.5 ± 2.99*	60.3 ± 3.64*,a	52.3 ± 6.33*a	21.6 ± 5.46*,a
120	92.4 ± 5.40*	61.9 ± 3.54*,a	62.3 ± 7.15*a	32.0 ± 6.46*,a
150	92.0 ± 4.10*	60.7 ± 3.21*,a	60.2 ± 7.61*a	38.0 ± 6.29*,a
180	101.3 ± 5.13*	58.9 ± 3.00*,a	65.9 ± 5.81*a	52.7 ± 8.53*,a
210	100.4 ± 4.98*	52.5 ± 3.04*,a	64.4 ± 6.08*a	55.9 ± 8.53*,a
240	95.8 ± 4.22*	47.1 ± 5.00*,a	67.1 ± 6.18*a	63.0 ± 7.54*,a
270	97.3 ± 4.87*	51.2 ± 3.06*,a	64.8 ± 7.11*a	70.4 ± 8.24*,a
300	93.3 ± 4.59*	51.5 ± 4.35*,a	65.3 ± 7.20*a	71.1 ± 8.49*
330	91.2 ± 5.75*	51.1 ± 3.97*,a	56.3 ± 8.49*a	72.8 ± 8.45*
360	85.6 ± 4.52*	45.0 ± 1.78*,a	55.3 ± 7.24*a	73.6 ± 8.22*
1440	40.3 ± 5.84	17.3 ± 2.82*,a	27.6 ± 4.19*	56.6 ± 7.55*

[a] Significantly different from control value at $P<0.05$.
* Significantly different from zero time (before induction) at $P<0.05$.

ing reasons. First, the presence of ethanol may help to extract some organic but polar 'active' solutes from the plant so that the extract would be more 'concentrated' for screening of pharmacological activities. Second, most traditionally used procedures were based on water extraction or by ingestion of whole plant as a source of salad; the 90% water present for extraction was nearest to traditional use. Third, after the extraction using 70% of ethanol the resulting extract was difficult to re-dissolve for activity screening. It is recognised that the amount of ethanol included in any extraction process should be screened according to traditional use and the suspected content of chemical groups in the plant extracts.

Traditional use of the flower of *P. grandiflora* for relieving toothache (personal communication from local healers) had not been confirmed by any scientific literature. However the aerial ground parts and seeds of *P. oleracea* have been reported as an anti-pyretic and analgesic agent (Rocha et al., 1994). Recently, in our laboratory, Islam et al. (1998) demonstrated the analgesic activity of the 10% ethanolic extract of aerial parts of *P. oleracea* var. *sativa* compared with the other two related species, *P. oleracea* and *P. grandiflora* using the hot plate and tail flick methods. The present study indicated that the 400-mg dose of the extract gave an earlier onset and higher and longer activity of analgesia than the 200-mg dose and the 4-mg diclofenac as active control after i.p. administration (Tables 2 and 3).

The leaves and the juice of the aerial parts of the plant have been used traditionally for the treatment of swelling and inflammation (Goldstein et al., 1976; Okwausaba et al., 1987; Al-Harbi et al., 1994). It was shown that *P. oleracea* var. *sativa*, *P. oleracea* and *P. grandiflora* studied plethysmometrically for their anti-inflammatory activity significantly reduced the increase in paw volume induced by carrageenan in rats (Parry et al., 1993). When compared with the active control, diclofenac, the 10% ethanolic extract (either 200 or 400 mg) prepared in our laboratories appeared to be longer acting after i.p. administration (Table 4).

Our previous observations on the central activities of the 10% ethanolic extract of *P. oleracea* var. *sativa*, show that these may also contribute to the analgesic properties of the extract. The prolonged onset time of pentylenetetrazole-induced convulsions and a significant dose-dependent decrease in the locomotor activity in mice suggested

an inhibitory action on the central nervous system (Radhakrishnan et al., 1998). Moreover, the administration of naloxone also nullified the analgesic activities of the 10% ethanolic extract of *P. oleracea* var. *sativa* (unpublished observation).

In conclusion, we have demonstrated, using conventional pharmacological models, the analgesic and anti-inflammatory properties of crude extract of the cultivar species of *P. oleracea* var. *sativa*. These observations support some of the traditional uses of the plant for medicinal purposes. More studies will be required for the purification of active chemical groups from the crude extracts and to ascertain the mechanisms of action of these crude extracts.

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Original Article

Portulaca oleracea extracts protect human keratinocytes and fibroblasts from UV-induced apoptosis

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Abstract: *Portulaca oleracea* extracts, known as Ma Chi Hyun in the traditional Korean medicine, show a variety of biomedical efficacies including those in anti-inflammation and anti-allergy. In this study, we investigate the protective activity of the *P. oleracea* extracts against UVB-induced damage in human epithelial keratinocytes and fibroblasts by several apoptosis-related tests. The results suggest that *P. oleracea* extracts have protective effects from UVB-induced apoptosis.

Key words: apoptosis – fibroblast – keratinocyte – *Portulaca oleracea* extracts – UVB

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Introduction

Ultraviolet radiation is an established environmental carcinogen, which has been implicated in the aetiology of human skin cancer. Because all UVC (<280 nm) and a large portion (90–99%) of UVB (280–320 nm) are absorbed by ozone in the stratosphere, UVA (320–400 nm) is the main irradiation reaching the surface of the earth. Despite a low presence of UVB, its high energy has positioned it as an important factor in photocarcinogenesis (1,2), suppression of the immune system (3) such as exacerbation of infections (4), and premature skin aging (5,6). Like other adverse agents (alkalating chemicals, oxidants), UVB induces changes in mammalian cell gene expression (7–9).

One of the hallmark events of UV-exposed skin is the formation of apoptotic keratinocytes, which die as sunburn cells within the epidermis. Biochemical analyses reveal that UVB-irradiation keratinocytes show DNA fragmentation accompanied by sequential caspase activation (10). Apoptosis is an active suicidal mechanism that is applied to eliminate unwanted or potentially deleterious cells under a variety of physiological and pathological circumstances, in multicellular organisms. It is generally accepted that induction of apoptosis following UV radiation is an important defensive mechanism to ensure the removal of irreversibly damaged and potentially carcinogenic cells. UVB-induced genomic DNA damage has been suggested to be a crucial event occurring at the commencement of the apoptotic programme. Furthermore, formation of ROS following UV radiation appears to be an additional factor in triggering the apoptotic machinery (11). As the keratinocytes were too seriously damaged for DNA repair to undergo apoptosis, the misregulation of UVB-induced apoptotic process may have a cardinal effect on the induction of skin cancer. Generally, the initiation of apoptosis is controlled by a regulation of the balance between death and survival signals recognized by a cell (12).

Portulaca oleracea is an annual succulent in the family Portulacaceae, which can reach 40 cm in height. About 40 varieties are currently cultivated. It has an extensive old-world distribution extending from North Africa through the Middle East and the Indian Subcontinent to Malaysia and Australasia. It is naturalized elsewhere and in some regions is considered an invasive weed. It has smooth, reddish, mostly prostrate stems and alternate leaves clustered at stem joints and ends. The yellow flowers have five regular parts and are up to 6 mm wide. The flowers appear depending upon rainfall and may occur year-round. The flowers open singly at the centre of the leaf cluster for only a few hours on sunny mornings. Seeds are formed in a tiny pod, which opens when the seeds are ready. Purslane has a taproot with fibrous secondary roots and is able to tolerate poor, compacted soils and drought. Known as Ma Chi Hyun in TKM (Ma Chi Xian in the traditional Chinese medicine), it is used to treat infections or bleeding of the genito-urinary tract as well as dysentery. The fresh herb may also be applied topically to relieve sores and insect or snake bites on the skin (13). Eating purslane can dramatically reduce oral lichen planus (14).

In this study, we have investigated the role of a *P. oleracea* extracts in UVB-induced apoptosis of human fibroblast and keratinocytes through DNA fragmentation, annexin V-FITC and TUNEL assay.

Materials and methods

Materials and equipment

Portulaca oleracea extracts were obtained from Bioland (Cheonan, Korea). Human fibroblast (CRL-2076) and human keratinocytes (CRL-2310) were purchased from American Type Culture Collection (ATCC; Manassas, USA), and media and reagents for the cell culturing were purchased from Invitrogen (Carlsbad, CA, USA), Abcam (Cambridge, UK), Sigma-Aldrich (St. Louis, MO, USA) and Nunc (Rochester, NY, USA). An ELISA reader (Tecan, Infinity M200; Mannedorf, Switzerland) and PCR (Bio-Rad, Mycycler thermal cycler; Hercules, California, USA) were used for the cytotoxicity assay.

Cell culture

Human normal fibroblast cells were cultured in Iscove's Modified Dulbecco's Media (IMDM) containing 10% foetal bovine serum (FBS) and 1% antibiotics at 37°C in a humidified atmosphere of 5% CO_2. The cells were then subcultured with 0.25% trypsin–0.53 mM EDTA after replacing with a fresh medium every 2 or 3 days.

Human keratinocytes were cultured in keratinocyte serum-free media (K-SFM) containing supplements at 37°C in a humidified atmosphere of 5% CO_2. The cells were then subcultured with 0.025% trypsin–EDTA solution after replacing with a fresh medium every 2 or 3 days.

Cell viability assay

Human fibroblasts were seeded in 24-well plates at a density of 1×10^5 cells per well and cultured at 37°C in 5% CO_2. Next, the culturing medium was exchanged for fresh serum-free medium, and the cells were allowed to incubate in a CO_2 incubator at 37°C in the presence of samples for 24 h. The cells were then treated with 100 μl of 2.5 mg/ml MTT (3-(4, 5-dimethyl- thiazol-2-yl)-2, 5-diphenyltetrazolium bromide) and incubated at 37°C for an additional 4 h. The medium containing MTT was discarded, MTT formazan produced was extracted with 1 ml of DMSO, and the absorbance was read at a wavelength of 570 nm. The level of cell viability was calculated as:

$$\text{Cell viability (\%)} = (OD_{570(\text{sample})}/OD_{570(\text{control})}) \times 100$$

where $OD_{570(\text{sample})}$ is the absorbance of the treated cells at 570 nm, and $OD_{570(\text{control})}$ is the absorbance of the negative control (non-treated cells) at 570 nm.

UVB irradiation

Cells were placed in culture plates and cultured in medium with supplements for 24 h. The medium was replaced by serum-free medium with various concentrations of *P. oleracea* extracts, and the cells were incubated for 1 h prior to irradiation with UVB. *Portulaca oleracea* extract-treated or untreated cells were rinsed twice with HBSS and exposed to UVB (350 mJ/cm^2 for fibroblasts, 50 mJ/cm^2 for keratinocytes), at the wavelength of 312 nm. After UVB exposure, the cells were replenished with serum-free medium including the *P. oleracea* extracts and followed up to 24 h at 37°C, 5% CO_2 incubator.

JC-1 staining

After 24 h, *P. oleracea* extract-treated and untreated cells were washed with PBS, and then the culture medium was replaced, without supplements, containing 1 μg/ml of JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-testraethylbenzimidazolyl carbo cyanine iodide). Then, the cells were incubated at 37°C for 1 h. The samples were protected from the light during the incubation. After the dye was removed, the cells were rinsed in PBS, and the serum-free medium was added. The cells were observed using a fluorescence microscope after the procedure.

Annexin V-FITC staining

At 24 h after UVB irradiation, *P. oleracea* extract-treated and untreated cells were stained with the reagents in the Annexin V-FITC apoptosis detection kit (ab14085; Abcam), as recommended by the manufacturer. Nuclei were counterstained using propidium iodide (PI), and the cells were analysed under a fluorescence microscope.

Analysis of DNA fragmentation by TUNEL assay and DNA ladder formation

TdT-mediated dUTP nick end Labeling (TUNEL) assay was performed using APO-BrdU TUNEL assay kit (Invitrogen). Cells were washed in PBS, incubated with TdT enzyme and BrdUTP in a water bath at 37°C for 1 h; cells were then washed and incubated (RT, 30 min) in the dark with fluorescein-conjugated anti-BrdU antibody. The nuclei were counterstained with propidium iodide and visualized with the Leica fluorescent microscope. For analysis of DNA fragmentation, DNA was isolated from the cells as described by Ling Wang et al. (15). 7.5 microgram of DNA was subjected to 1.5% agarose gel electrophoresis.

Results and discussion

Cytotoxicity of *P. oleracea* extracts with MTT assay

In the cell viability assay (MTT assay) in human fibroblast and keratinocytes, *P. oleracea* extracts were found to have no cytotoxicity (Fig. 1).

Mitochondrial membrane depolarization detection by JC-1 staining

Loss of the mitochondrial inner transmembrane potential is often associated with the early stages of apoptosis and may be one of the central features of the process (16). Collapse of this potential is thought to coincide with the opening of the mitochondrial permeability transition pores, allowing passage of ions and small molecules. The resulting equilibration of ions leads in turn to the decoupling of the respiratory chain and subsequently to the release of cytochrome *c* into the cytosol (17,18). JC-1 is a cationic dye that exhibits potential-dependent accumulation on mitochondria, indicated by a fluorescence emission shift from green (~525 nm) to red (~590 nm). Figure 2 is a JC-1 staining image of *P. oleracea* extracts with and without UVB. In Fig. 2a–d, on fibroblast, the UVB-treated cells were showing green colour, which means the cells were disrupted of the mitochondrial membrane potential by UVB (Fig. 2b). On the other hand, as treated with *P. oleracea* extracts before and after UVB irradiation, increase in the red to green fluorescence intensity ratio showed dose dependently (Fig. 2c,d). It was the same on the keratinocytes (Fig. 2e–h).

Phosphatidylserine exposure detection by annexin V-FITC staining

In viable cells, phosphatidylserine is located on the cytoplasmic surface of the cell membrane. As cells undergo apoptosis, phosphatidylserine is translocated to the outer leaflet of the plasma membrane and exposed to the extracellular environment (19). The human vascular anticoagulant, annexin V, is a Ca^{2+}-dependent phospholipid-binding protein that has a high affinity for phosphatidylserine (20). Thus, annexin V labelled with a fluorophore or biotin can identify apoptotic cells by binding to phosphatidylserine exposed on the outer leaflet (21). UVB irradiation-induced apoptosis on fibroblast showing strong green fluorescence by ann-

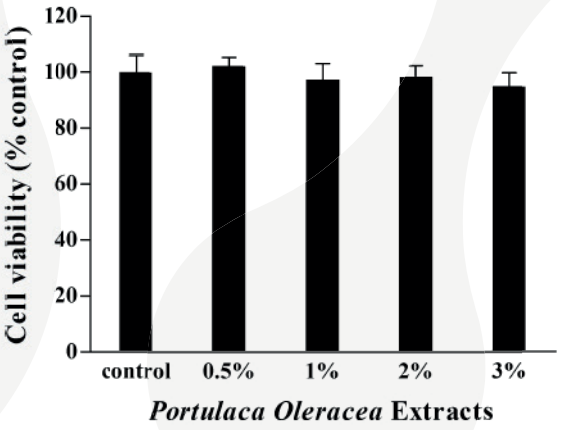

Figure 1. Cytotoxicity of *Portulaca oleracea* Extracts. The data are expressed as mean values (±standard deviation) of three experiments.

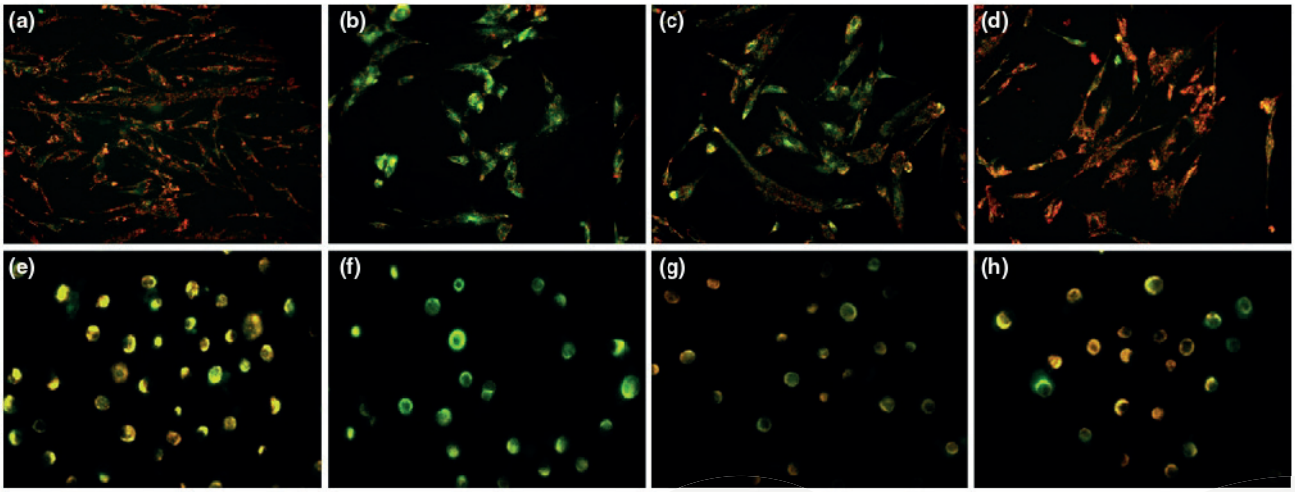

Figure 2. Mitochondrial Membrane Depolarization Detection by JC-1 Staining. The figures indicate the effects of *Portulaca oleracea* extracts on apoptosis in human fibroblast (a–d) and keratinocytes (e–h). (a) is UVB-untreated fibroblast, and the others are 350 mJ/cm^2 of UVB-treated fibroblast groups (b: without POE, c: with 1% of *P. oleracea* extracts and d: with 2% of *P. oleracea* extracts). (e) is UVB-untreated keratinocytes, and (f–h) are 50 mJ/cm^2 of UVB-treated keratinocytes (f: without POE, g: with 1% of *P. oleracea* extracts and h: with 2% of *P. oleracea* extracts).

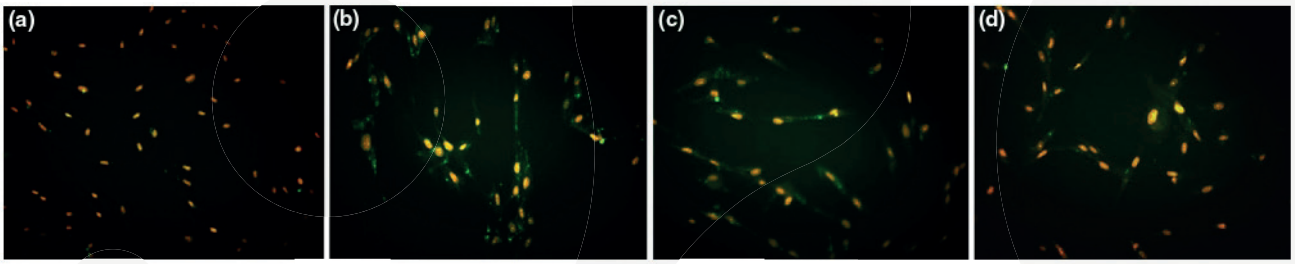

Figure 3. Phosphatidylserine Exposure Detection by Annexin V-FITC Staining. (a) is UVB-untreated fibroblast, and the others are 350 mJ/cm^2 of UVB-treated groups (b: without POE, c: with 1% of *Portulaca oleracea* extracts and d: with 2% of *P. oleracea* extracts).

exin-V-labelled FITC binds to phosphatidylserine exposed on the outer leaflet (Fig. 3b). However, the higher the concentration of the *P. oleracea* extract, the lower was the intensity of the green fluorescence (Fig. 3c,d). These results indicated that *P. oleracea* extracts have cell protection effects against the UVB-induced apoptosis.

Detection of apoptotic DNA fragmentation by TUNEL assay and DNA ladder formation

To further investigate the protective effects of *P. oleracea* extracts against UVB-induced apoptotic cell death, DNA fragmentation assay was performed to confirm that DNA fragmentation occurs or not by TUNEL staining and gel electrophoresis. DNA fragmentation assay is among the most reliable methods for the detection of apoptotic cells (22). In TUNEL assay, when DNA strands are cleaved or nicked by nucleases, 3′-hydroxyl ends are exposed. The hydroxyl groups can then serve as a starting point for terminal deoxynucleotidyl transferase (TdT), which adds deoxyribonucleotides in a template-independent fashion. Addition of BrdUTP to the TdT reaction thus provides a means to label the DNA strand breaks. Once incorporated into DNA, the BrdU can be detected by an anti-BrdU antibody using standard immunohistochemical techniques. Another method of detecting DNA fragmentation is the observation of DNA ladder formation by gel electrophoresis. DNA cleavage between nucleosomes during apoptosis results in 200–5000 base pair fragments referred to as ladders. As shown in Fig. 4, apoptotic nuclei and DNA strand breakages were clearly observed in *P. oleracea* extract-untreated cells at 24 h following UVB irradiation (B). The DNA cleavage, however, was reduced in *P. oleracea* extract-treated cells (C, D), which was also shown on the keratinocytes (Fig. 4e–h). In Fig. 5a, the characteristic ladder formation was recognized in the isolated DNA obtained from the fibroblast cells that were irradiated at a UVB dose of 350 mJ/cm^2 (lane 2). Treatment with *P. oleracea* extracts before and after UVB irradiation suppressed DNA ladder formation in a concentration-dependent manner. It was also shown on the keratinocytes at a UVB dose of 50 mJ/cm^2 (Fig. 5b). These data indicated that *P. oleracea* extracts protected human skin cells from UVB-induced cell damage.

Chronic repeated exposures (~high extent of exposure) to sunlight are epidemiologically shown to be the main cause of skin cancers. UVB is experimentally demonstrated to be the most significant light to induce skin cancer (11). It is generally accepted that induction of apoptosis following UV radiation is an important defensive mechanism to ensure the removal of irreversibly damaged and potentially carcinogenic cells. Although apoptosis is an active mechanism to eliminate potentially deleterious cells

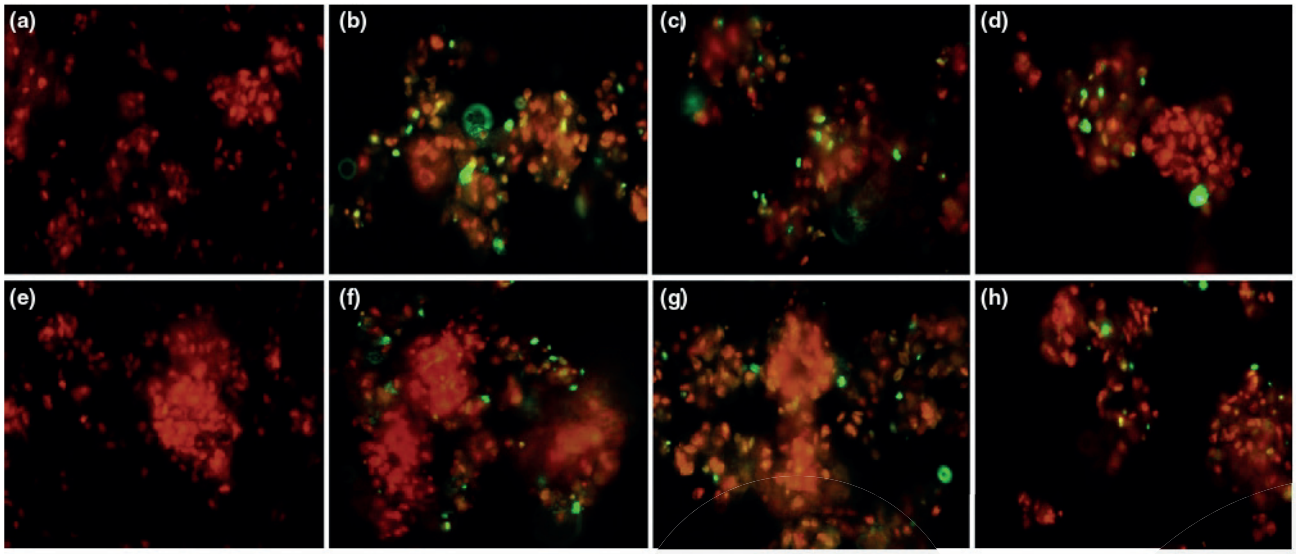

Figure 4. DNA Fragmentation Detection by TUNEL Assay. (a) is UVB-untreated fibroblast, and the others are 350 mJ/cm^2 of UVB-treated fibroblast groups (b: without POE, c: with 1% of *Portulaca oleracea* extracts and d: with 2% of *P. oleracea* extracts). (e) is UVB-untreated keratinocytes, and (f–h) are 50 mJ/cm^2 of UVB-treated keratinocytes (f: without POE, g: with 1% of *P. oleracea* extracts and h: with 2% of *P. oleracea* extracts).

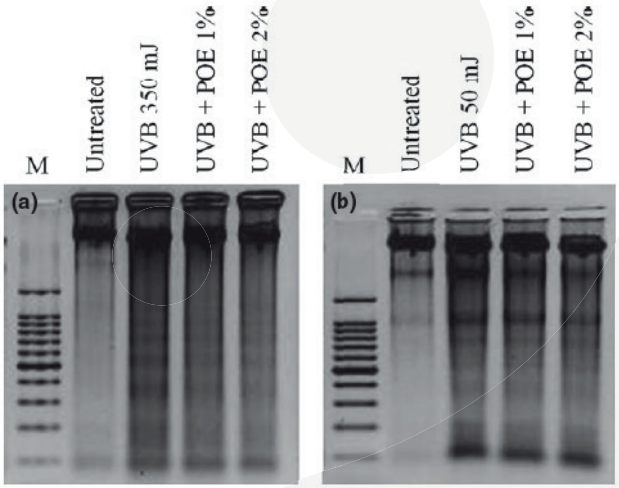

Figure 5. DNA Fragmentation Detection by DNA Ladder Formation. DNA was extracted from *Portulaca oleracea* extract-treated or untreated cells with or without UVB irradiation. DNA was electrophoresed on 1.5% agarose gel. (a) is on fibroblast, and (b) is on keratinocytes. (M: 100 bp DNA marker).

under a variety of physiological and pathological circumstances (12), UVB-induced genomic DNA damage has also been suggested to be a crucial event occurring at the commencement of skin aging. Age-related accumulation of DNA damage appears to randomly affect the genome, but some chromosomal regions, such as telomeres, are particularly sensitive to age-related deterioration (23). Hayflick demonstrated that a population of normal human foetal cells in a cell culture divides between 40 and 60 times, which is called as the Hayflick limit, and the population then enters a senescence phase (~aging phase) (24). Afterwards, the Hayflict limit, the limited proliferative capacity of some types of *in vitro*-cultured cells, was explained by telomere exhaustion (25). It is accepted that telomere exhaustion contributes to organismal aging at least by impairing cell proliferation and viability (26). Thus, we hypothesized that when UVB-induced apoptotic cell death occurs, cells adjacent to dead cells will proliferate to maintain homeostasis so that apoptosis can expedite telomere exhaustion of cells and accelerate aging in skin. Furthermore, preventing DNA damage is an important issue in the anti-aging. Therefore, we investigated the role of a *P. oleracea* extracts in UVB-induced apoptosis of human fibroblast and keratinocytes through DNA fragmentation, annexin V-FITC and TUNEL assay. Taking together, our findings indicate that *P. oleracea* extracts have cell protection effects against the UVB-induced apoptosis.

Conclusions

Portulaca oleracea extracts (Ma Chi Hyun in TKM) are used to treat infections or bleeding of the genito-urinary tract as well as dysentery. The fresh herbs may also be applied topically to relieve sores and insect or snake bites on the skin (13). In this study, we showed that *P. oleracea* extracts protected human fibroblast and keratinocytes cells from UVB-induced damage *in vitro*. In particular, *P. oleracea* extracts effectively reduced cell death and apoptotic DNA cleavage after UVB irradiation. According to our data, *P. oleracea* extracts may protect human skin cells from UVB-induced apoptosis. Thus, it is suggested that *P. oleracea* extracts may be used as an effective cosmetic ingredient to prevent UVB-induced skin damage.

Conflict of interest

The authors declare that they have no conflict of interests.

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