

## Screening of Sun Protective Activity of the Ethyl Acetate Extracts of Some Medicinal Plants

Payam Khazaeli<sup>a</sup> and Mitra Mehrabani<sup>b\*</sup>

<sup>a</sup>Department of Pharmaceutics, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran. <sup>b</sup>Department of Pharmacognosy, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.

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### Abstract

Natural substances extracted from plants have recently been considered as potential sunscreen resources because of their ultraviolet ray absorption in the UVA region and their antioxidant activity. In the present study, the UV protective effects of ethyl acetate extracts of some common medicinal plants, which have flavonoids and other phenolics as the most important components, were evaluated using diffuse transmittance method and calculating Sun Protection Factors (SPFs). The ethyl acetate extracts of sixteen medicinal plants were analyzed for their in vitro SPF by measurement of their transmittance at different concentrations in methanol.

Extracts of leaves of *Dracocephalum moldavica* L. (Lamiaceae) and flowering tops of *Viola tricolor* L. (Violaceae) had the highest SPFs, i.e. 24.79 and 25.69, respectively.

*D. moldavica* and *V. tricolor* had high amounts of phenolic compounds and flavonoids, which could be the cause for their high SPF.

**Keywords:** Medicinal plants; Sun Protection Factor; Ethyl acetate extract.

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### Introduction

While there is little literature on the ways by which people protected themselves against the sun, evidence from paintings suggests that clothes covering the body, veils and large brim hats were used by ancient Greeks, and umbrellas were used in ancient Egypt, Mesopotamia, China and India. In 1889, Widmark used acidified quinine sulfate to absorb UVB. Apparently due to the fact that quinine fluoresces when irradiated with UV, he rightly assumed that it would absorb the short wavelengths. In 1891, Hammer repeated Widmark's experiments using quinine prepared in the form of a lotion or ointment as the first human sunscreen. At the turn of the century,

various plant extracts had been used in folk medicine (1).

Sun radiation constantly impacts the earth with approximately 50% visible light (400-800 nm), 40% infrared radiation (IR) (1300-1700 nm), and 10% ultraviolet radiation (UV) (10-400 nm). UV is divided conventionally to UV-A (320-400 nm), UV-B (290-320 nm), UV-C (100-290 nm), and vacuo UV (10-100 nm) (2).

Sunscreens are chemicals that provide protection against the adverse effects of solar and in particular UV radiation (3). Natural substances extracted from plants have been recently considered as potential sunscreen resources because of their ultraviolet ray absorption in the UV region (4) and their antioxidant activity (5). Green tea polyphenols, Aloe barbadensis extract, aromatic compounds isolated from lichens and

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\* Corresponding author:

E-mail: mmehrabani@hotmail.com

several glycosides of aesculin are examples of natural substances evaluated for their sunscreen properties (1, 3, 6, 7).

There is strong evidence that DNA-damaging ultraviolet (UV) light induces the accumulation of UV light-absorbing flavonoids and other phenolics in dermal tissue of the plant body. This suggests physiological function, yet speculative in light protection in plants and of course in human (5, 8). Ethyl acetate could particularly extract flavonoids and other phenolics from plants (9).

In the present study, we evaluated the UV protective activities of ethyl acetate extracts of some most available and common medicinal plants which have flavonoids and other phenolics as the most important chemicals (10, 11) using diffuse transmittance method and calculating Sun Protection Factors (SPFs).

## Experimental

### Plants

Different parts of sixteen medicinal plants were collected from different regions of Iran. The specimens were prepared and authenticated (Table 1). The herbal samples were then kept at the herbarium of Faculty of Pharmacy, Kerman University of Medical Sciences.

### Extraction procedure

The air-dried herbal materials were ground into fine powders and extracted by maceration method two times with 90% and 50% hydro-methanol solutions respectively (12). After filtration of total extracts, the solvent was removed under reduced pressure to give the gummy mass. Then these residues were dissolved in 20% hydro-methanol solution and kept in refrigerator for 48 h until the chlorophyll was precipitated. The extracts were filtrated and extracted with ethyl acetate in decanter until the ethyl acetate was exactly uncolored (9). These extracts were dried under reduced pressure and the yield values were determined.

### Screening of sun protection activity by determination of the *in vitro* sun protection factor (SPF)

The ethyl acetate extracts of plants were

analyzed for the *in vitro* SPF. The extracts were dissolved in methanol UVsolv (Merck) (13, 14). Scanning spectra of the samples in solution were obtained by running from 337.5 to 292.5 nm (at five nm intervals). The SPF determination model used in this study based on the following equation proposed by Gharavi *et al.* (15):

$$SPF = \frac{\sum_{292.5}^{337.5} E(\lambda) \epsilon(\lambda)}{\sum_{292.5}^{337.5} E(\lambda) \epsilon(\lambda) T(\lambda)}$$

where:  $T(\lambda)$  is the measured sunscreen transmittance at  $\lambda$ ;  $E(\lambda)$  is the spectral irradiance of terrestrial sunlight at  $\lambda$ ; and  $\epsilon(\lambda)$  is the erythemal action spectrum at  $\lambda$ .

The values of the  $E(\lambda)$  and  $\epsilon(\lambda)$  were calculated according to the report by Gharavi *et al.* (15).

$T(\lambda)$  was measured four times for each extract and the mean was used for calculation of SPF. Three different concentrations of each extract (according to Beer's Law) were used for obtaining the standard curve and calculating SPF in 2 mg/ml solution according to Gharavi *et al.* method (15).

## Results and discussion

Table 1 shows the SPFs of 2 mg/ml in solutions of extracts methanol. Extracts of leaves of *Dracocephalum moldavica* L. (Lamiaceae) and flowering tops of *Viola tricolor* L. (Violaceae) had the highest SPFs, i.e. 24.79 and 25.69 respectively. SPFs of methanol solutions of flowers of *Calendula officinale* L. (Asteraceae) and flowering tops of *Hypericum perforatum* L. (Hypericaceae) were 12.01 and 12.21 respectively. Sun protective activities of the rest of the plants were low representing by their sun protection being lower than 10.

In this study, among the sixteen plants studied, only *Dracocephalum moldavica* and *Viola tricolor* had sun protective activity, considering the SFP values actors of higher than 20.

Most spectrophotometric techniques for transmittance measurements rely on preparing samples with a uniform and known thickness

Table 1. Sun Protection Factors (SPF) of ethyl acetate extracts of sixteen medicinal plants.

No.	Scientific name (Family)	Plant name	Place and date of collection (number of voucher specimens)	Part of use	% total methanol dry extract (m/w)	% ethyl acetate dry extract (m/w)	SPF of 2 mg/ml of methanol solution (FDA standard)
1	<i>Elysiya rosea</i> (L.) Merrill (Fabaceae)	Soya	Haft, Kermanshah, Aug 2004 (1304)	Seeds (defatted)	3	1	4.66
2	<i>Glycyrrhiza glabra</i> L. (Fabaceae)	Shirin-bayun	Haft, Kermanshah, Sep 2004 (1305)	Roots	3	2	2.82
3	<i>Melilotus iranicus</i> L. (Rutaceae)	Hakimabad-shirvan	Darbuz, Tehran, May 2004 (1304)	Flowers	5	3	3.41
4	<i>Ziziphora japonica</i> Mill. (Rutaceae)	Arak	Hajjabad, Kermanshah, Sep 2004 (1305)	Fruits	5	1	4.80
5	<i>Ziziphora spinosa-chalcidica</i> (L.) Winkl. (Rutaceae)	Soleh	Shahrabad, Kermanshah, Aug 2004 (1305)	Leaves	6	3	4.64
6	<i>Crotophyaga nemoralis</i> A. Pajarek (Rutaceae)	Zahabik-e-varesh	Esfahan, Kermanshah, Aug 2005 (1305)	Leaves	5	2	2.15
7	<i>Dioscorea bulbifera</i> L. (Dioscoreaceae)	Hakimabad	Kermanshah, July 2005 (1305)	Leaves	3	1	24.79
8	<i>Polygonum aviculare</i> L. (Polygonaceae)	Shahrabad	Kermanshah, May 2005 (1305)	Leaves	4	1	4.82
9	<i>Ruscus officinalis</i> L. (Liliaceae)	Kermanshah	Kermanshah, June 2005 (1306)	Leaves	4	2	2.51
10	<i>Trigonotis foeniculum-galeatum</i> L. (Fabaceae)	Shahrabad	Kermanshah, June 2005 (1306)	Leaves	2	1	5.14
11	<i>Colubella officinalis</i> L. (Rutaceae)	Hakimabad-shirvan	Kermanshah, May 2006 (1306)	Flowers	2	1	12.01
12	<i>Centaurium cyaneum</i> L. (Rutaceae)	Gol-e-gardan	Haft, Kermanshah, July 2006 (1307)	Flowering tops	7	3	3.63
13	<i>Hypocistis purshiana</i> L. (Hydrocaraceae)	Arak-e-chai	Isfahan, July 2006 (1306)	Flowering tops	6	2	12.21
14	<i>Lamium album</i> DC. (Lamiaceae)	Lamad	Esfahan, June 2006 (1306)	Flowering tops	7	4	4.97
15	<i>Lamium album</i> L. (Lamiaceae)	Hafsh	Shahrabad, Kermanshah, July 2006 (1307)	Leaves	6	4	2.40
16	<i>Vitis rotundifolia</i> L. (Vitaceae)	Hakimabad-shirvan	Kermanshah, April 2006 (1306)	Flowering tops	5	2	21.89

FDA: Food and Drug Administration

so that the optical path length through the sample is standardized. Many samples are dissolved in special solvents and placed into 10 mm path length cuvettes. The cuvettes for UV spectrophotometry are usually made from quartz (fused silica) which is transparent to UV wavelengths (14).

In the Solvent method, different concentrations of test products in methanol were prepared. Each sample's transmittance was measured to evaluate the SPF value.

Sunscreens contain a wide variety of chemicals that have specific absorbance in some parts of the UV spectrum. There are very few single chemical substances that have absorbance over the full range of UV. This property is needed for a product to be considered as a proper wide-spectrum sunscreen. Plant extracts, due to containing a wide range of natural compounds, usually cover this full range of UV wavelengths. One approach to protecting the body from the harmful effects of UV irradiation is to use active photoprotectives. In recent years, naturally occurring compounds have gained considerable attention as protective agents (16).

There is a review about the photoprotective effects of some naturally occurring herbal polyphenols and phenolic-rich extracts in studies of the skin damage induced by UV irradiation. Several studies have shown the flavonoids to act as scavengers of superoxide anions, singlet oxygen, hydroxyl radicals, and lipid peroxy radicals. There are also reports of flavonoids inhibiting the activities of many enzymes, including lipoxygenase, cyclooxygenase, monooxygenase, xanthinase, mitochondrial succinate dehydrogenase and NADH-oxidase, phospholipase A<sub>2</sub>, protein kinases, and nuclear transcription factor. Phenolics are believed to be capable of acting in redox-sensitive signalling cascades to inhibit DNA damage. In contrast to their beneficial effects, some phenolics however have also been found in vitro to be mutagenic. These harmful effects are suspected to result from the prooxidant rather than antioxidant action of these compounds. Therefore, it is necessary to investigate their toxic effects before human use. In the past decade, the antioxidant activity of herbal phenolics, namely phenolic acids and flavonoids, has been given much

attention. Many flavonoids such as quercetin, luteolin and catechins are better antioxidants than the nutrients vitamin C, vitamin E and  $\beta$ -carotene. Therefore, the phenolics may be beneficial in preventing UV-induced oxygen free radical generation and lipid peroxidation, i.e. events involved in pathological states such as photoaging and skin cancer (16).

Topical application of extract of flower buds of *Capparis spinosa* reduces UV-induced skin erythema in healthy human volunteers. Topical application of *Culcitium reflexum* extract in the form of a gel has proven to be a significant in vivo protection against UV-induced skin erythema in healthy human volunteers. *Ginkgo biloba* extract has also been found to decrease the number of UVB-induced sunburn cells in mice skin. Oral administration of *G. biloba* extract leads to increased super-oxide dismutase activity in UVB-irradiated mice skin. Extract of *Krameria triandra* root has shown protective effects against UVB-induced photodamage in human keratinocyte cells. The extract of *Prunus persica* flower was also shown to delay tumour development after UVB-induced skin carcinogenesis in SKH-1 hairless mice. It also reduces UVB-induced ear edema in IRC mice. The lyophilised flavonolic fraction of *Sedum telephium* leaf extract appears to possess potent protective effects against UV-induced skin erythema in human volunteers (16).

The *Prunella vulgaris* extract demonstrated a concentration-dependent photoprotection (17). In vitro SPF value for the crude *Pothomorphe umbellata* root extract was 21.53 (13).

*Dracocephalum moldavica* is an annual plant native in Central Asia and naturalized in Eastern and Central Europe where it is used in folk medicine as antiseptic and stimulating remedy. It contains high amounts of phenolic compounds such as: rosmarinic acid: 2.84 g%, caffeic acid: 50.3 mg%, total flavonoids: 0.58 g% ( apigenin, luteolin flavon aglycons), and tannins: 12.9 g% (18).

*Viola tricolor* is an annual plant indigenous to Eurasia region. It is traditionally used for bronchitis and rheumatism. *V. tricolor* is an especially valued remedy for treating skin diseases. Used both internally and topically, it is good for eczema, psoriasis and acne. The

active compounds are flavonoids, including violanthin, rutin, violaquercitrin and salicylates. Both salicylates and rutin contained in the plant are anti-inflammatory agent. Due to the high concentration of rutin in the flowers, this plant may be used to prevent UV-induced oxygen free radical generation (19, 20).

*D. moldavica* and *V. tricolor* had high amounts of phenolics and flavonoids. The high value of SPF (24.79 and 25.69 respectively) could be due to these chemicals.

For following studies, it is suggested to prepare topical sunscreen formulations from these two extracts and measure their SPFs.

### Acknowledgement

This work was supported by a grant from the Research Council of Kerman University of Medical Sciences, Kerman, Iran.. We would like to thank Dr. S. Derafsh, Dr. A. Abedini, Dr. Sh. Ahadpour and Dr. S.M. Majd-zadeh for the technical works.

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