

Original Research Article

Sun protection factor, total phenol, flavonoid contents and antioxidant activity of medicinal plants from Iran

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Abstract

Purpose: To evaluate the correlation between sun protection factor (SPF) and the content of phenol and flavonoid and antioxidant activity.

Methods: Different parts of 9 medicinal plants were extracted with methanol using three extraction methods (percolation, Soxhlet and ultrasonically assisted extraction) to obtain 42 crude extracts. Their phenol and flavonoid contents, and antioxidant activities were determined using Folin-Ciocalteu reagent, aluminum chloride method and DPPH radical-scavenging activity, respectively. The SPF values were determined and correlated with the phenol and flavonoid contents as well as antioxidant activities.

Results: The phenol and flavonoid contents, and antioxidant activities ranged from 54.16-688.97mg GAE/g, 13.38-146.60 mg QE/g and 9.5-1472.4 µg/mL, respectively while the SPFs were between 0.067 and 0.841. The highest SPF was related to *Cucumis melo* L. ultrasonically assisted leaf extract (0.841) and *Artemisia absinthium* L., aerial parts extracted with percolation method (0.717). A significant correlation was found between SPF and phenolic ($p=0.003$) and flavonoid contents ($p=0.023$).

Conclusion: This study showed a correlation between SPF and phenolic and flavonoid contents. Ultrasonically assisted extract of *C. melo* leaf has suitable SPF and can be used in sun screen formulations.

Keywords: *Artemisia absinthium*, *Cucumis melo*, *Echium amoenum*, *Orobancha orientalis*, *Sambucus ebulus*, Sun burn, Sun protection factor, flavonoids, phenols

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INTRODUCTION

About one million people are diagnosed with skin cancer every year [1]. Exposure to solar radiation has negative effects on the skin [2]. Ultraviolet light is mainly responsible for skin damage [1]. Sunscreens absorb or block UV rays of sunlight. There is some information regarding possible adverse reaction by synthetic sun-screen

products [3]. There is little published data describing the use of herbal sunscreens and their sun protection factor (SPF) [4].

Natural substances have been recently considered as potential sources for agents with sunscreen properties [5] because of their absorption in the UV region [6] and their antioxidant activity [7]. Green tea polyphenols,

aromatic compounds from lichens, glycosides of *aesculin* and *Murraya koenigii* are examples of natural substances evaluated for their sunscreen properties [8-10]. UV radiation increases oxidative stress in skin cells leading to cancer initiation and promotion. There has been an increasing interest in the use of antioxidants in sunscreens to provide supplemental photo protective action. Antioxidants from natural sources may provide new possibilities for the treatment and prevention of UV mediated diseases [7].

The Iranian flora is rich in medicinal plants with a high potential for providing these antioxidants. Among them, *Sambucus ebulus*, *Artemisia absinthium* and *Cucumis melo* are three medicinal plants with high antioxidant activities [11-13]. There are very limited reports on correlation between phenol/flavonoid contents or antioxidant activity and their SPF values [14].

The goals of this research were to evaluate: 1) SPF of 42 extracts from nine medicinal plants; 2) the phenol and flavonoid contents of the plants; 3) the antioxidant activities of the plants and 4) the correlation between SPF and the content of phenol and flavonoid and antioxidant activity.

EXPERIMENTAL

Plant material and preparation of freeze-dried extract

Cucumis melo L. (Cucurbitaceae), aerial parts, leaf and fruit, *Sambucus nigra* L. (Caprifoliaceae), leaf, fruit and bark, *Artemisia absinthium* L. (Asteraceae), and *Orobanchae orientalis* G. Beck (Orobanchaceae), aerial parts, *Vicia faba* L. (Fabaceae), bean and hall, *Albizia julibrissin* Durazz. (Fabaceae/Leguminosae), leaf and flower, *Danae racemosa* Moench. (Liliaceae), leaf, *Sambucus ebulus* L. (Caprifoliaceae), flower and *Echium amoenum* Fisch and CA Mey. (Boraginaceae), petals were obtained in spring and summer, from Sari, Iran. Samples were authenticated by Dr Bahman Eslami and their voucher specimens were deposited (no. 1017-1025) in Sari School of Pharmacy herbarium. The plant materials were allowed to dry at room temperature for 2 weeks, and milled to obtain 2-3 mm particles for incubation with methanol to obtain methanolic extracts. Extracts were separated from the sample residues by filtration using filter paper and the process was repeated three times. The resulting extracts were concentrated using a rotary evaporator under vacuum at 36 ± 2 °C and the crude solid extracts were freeze-dried for complete solvent removal.

Ultrasonically-assisted extraction

Samples were extracted with methanol in an ultrasonic bath by indirect sonication (frequency of 60 kHz) at 25 ± 2 °C for 1 h. The extracts were then separated from the samples residue by filtration. Resultant extracts were concentrated in a rotary evaporator until a crude solid extracts were obtained, which then freeze-dried for complete solvent removal [15].

Soxhlet-assisted extraction

Powdered samples were incubated with methanol for 24h in a Soxhlet extractor. The extracts were concentrated in a rotary evaporator to completely remove the solvent.

Determination of total phenolic and flavonoid contents

Total phenolic content was measured colorimetrically using Folin-Ciocalteu reagent [15]. The results were expressed as gallic acid equivalents (GAE). Total flavonoids were estimated using $AlCl_3$ method [16] and the contents calculated based on quercetin as standard from a calibration curve.

Evaluation of DPPH radical-scavenging activity

1,1-Diphenyl-2-picryl hydrazyl radical (DPPH) was used to assay free radical-scavenging activity of the extracts [17]. To 1ml of each extract (different concentrations, ranging from 25-800 μ g/mL) 1ml methanol solution of DPPH (100 μ M) was added. After 15 min incubation at room temperature in the dark, the absorbance was recorded at 517 nm. The experiment was done in triplicates using vitamin C and butylated hydroxyl anisole (BHA) as positive controls.

Computation of *in vitro* SPF

The extracts were dissolved in methanol and scanned in the range of 337.5 to 292.5 nm (at five nm intervals) as previously described [18]. Screening of sun protection activity was then measured by determination of *in vitro* SPF based on the following equation proposed by Gharavi et al [19]:

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

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

values were calculated as described previously [19]. For each extract, T(λ) was measured three times and the means was used for SPF calculation. At least five different concentrations of each extract were used to obtain the standard curve and in calculating SPF in 2 mg/mL solution.

RESULTS

The antioxidant activity of extracts calculated using DPPH method is presented in Table 1. Best result was that of *S. ebulus* flower extract obtained by percolation method ($IC_{50} = 9.5 \pm 0.9$

$\mu\text{g/mL}$). *S. ebulus* flower extract (prepared using Soxhlet extractor, $IC_{50} = 9.8 \pm 0.4 \mu\text{g/mL}$), *O. orientalis* aerial part extract (prepared using ultra sonication, $IC_{50} = 17.9 \pm 0.8 \mu\text{g/mL}$), *A. julibrissin* leaf extract (prepared using ultra sonication, $IC_{50} = 21.6 \pm 0.6 \mu\text{g/mL}^{-1}$) and *S. nigra* leaf extract (prepared using ultra sonication, $IC_{50} = 21.6 \pm 0.8 \mu\text{g/mL}$) showed satisfactory degrees of antioxidant activity. These extracts all had a good antioxidant activity compared to the positive controls, vitamin C ($IC_{50} = 3.7 \pm 0.1 \mu\text{g/mL}$) and BHA ($IC_{50} = 29.3 \pm 5.9 \mu\text{g/mL}$).

Table 1: Sun protection factor, total phenol contents, total and flavonoid contents and antioxidant activities of 42 tested extracts

Plant name	Extraction method	Part used	Extraction yield (%)	Phenol contents (GAE /g of extract)	Flavonoids contents (QE /g of extract)	Antioxidant activity (IC_{50})	SPF (at 2 mg ml ⁻¹)
<i>C. melo</i>	Ultrasonic	Leaf	13.4	72.41 \pm 1.5	19.47 \pm 1.1	582.7 \pm 12.7	0.841 \pm 0.02
	Ultrasonic	Fruit	20.5	88.62 \pm 3.7	16.96 \pm 0.4	780.1 \pm 19.6	0.169 \pm 0.01
	Percolation	Leaf	15.6	71.20 \pm 2.5	18.69 \pm 0.9	115.0 \pm 3.7	0.689 \pm 0.02
	Percolation	Fruit	21.9	77.57 \pm 1.3	13.38 \pm 1.2	975.6 \pm 31.5	0.299 \pm 0.01
	Soxhlet	Leaf	16.6	111.77 \pm 3.4	59.48 \pm 2.9	445.3 \pm 10.3	0.205 \pm 0.01
	Soxhlet	Fruit	30.8	80.92 \pm 2.1	78.88 \pm 3.2	643.8 \pm 22.8	0.144 \pm 0.00
<i>S. nigra</i>	Ultrasonic	Leaf	18.5	185.24 \pm 6.3	58.56 \pm 2.4	21.6 \pm 0.8	0.245 \pm 0.01
	Ultrasonic	Fruit	14.5	71.98 \pm 2.1	42.53 \pm 1.3	548.4 \pm 15.5	0.446 \pm 0.02
	Ultrasonic	Bark	3.2	100.86 \pm 3.7	38.63 \pm 0.8	193.1 \pm 8.7	0.311 \pm 0.01
	Percolation	Leaf	8.5	132.34 \pm 3.4	109.48 \pm 7.1	1472.4 \pm 32.1	0.245 \pm 0.01
	Percolation	Fruit	17.5	99.32 \pm 2.9	19.62 \pm 1.5	1227.3 \pm 39.7	0.159 \pm 0.00
	Percolation	Bark	2.4	168.96 \pm 4.7	81.43 \pm 2.7	831.4 \pm 24.1	0.365 \pm 0.02
	Soxhlet	Leaf	11.4	115.86 \pm 5.1	101.94 \pm 2.9	769.7 \pm 19.8	0.285 \pm 0.01
	Soxhlet	Fruit	3.2	158.62 \pm 4.6	45.60 \pm 1.1	844.6 \pm 18.9	0.086 \pm 0.00
	Soxhlet	Bark	4.2	159.13 \pm 6.3	33.06 \pm 0.7	845.9 \pm 21.1	0.333 \pm 0.01
	Soxhlet	Leaf	11.4	115.86 \pm 5.1	101.94 \pm 2.9	769.7 \pm 19.8	0.285 \pm 0.01
<i>V. faba</i>	Ultrasonic	Bean	6.6	54.16 \pm 2.5	27.59 \pm 1.1	918.7 \pm 34.5	0.067 \pm 0.00
	Ultrasonic	Hull	9.12	103.38 \pm 3.3	33.62 \pm 1.3	56.9 \pm 2.5	0.083 \pm 0.00
	Percolation	Bean	14.6	69.16 \pm 2.8	22.59 \pm 0.9	1143.7 \pm 40.2	0.057 \pm 0.00
	Percolation	Hull	32.3	78.62 \pm 3.6	62.75 \pm 1.4	85.8 \pm 3.8	0.174 \pm 0.01
	Soxhlet	Bean	12.4	56.57 \pm 1.4	28.45 \pm 1.1	697.0 \pm 23.3	0.104 \pm 0.01
	Soxhlet	Hull	39.5	110.25 \pm 6.1	60.52 \pm 2.5	87.3 \pm 3.4	0.093 \pm 0.00
<i>A. absinthium</i>	Ultrasonic	Aerial	16.5	171.77 \pm 5.8	136.25 \pm 5.5	364.1 \pm 14.7	0.606 \pm 0.02
	Percolation	Aerial	25.4	188.58 \pm 4.6	113.00 \pm 5.3	345.1 \pm 16.4	0.717 \pm 0.02
	Soxhlet	Aerial	28.4	220.69 \pm 6.3	136.45 \pm 4.9	191.6 \pm 7.2	0.346 \pm 0.01
<i>A. julibrissin</i>	Ultrasonic	Leaf	15.4	565.52 \pm 22.8	139.66 \pm 6.2	21.6 \pm 0.6	0.247 \pm 0.01
	Ultrasonic	Flower	12.7	340.35 \pm 14.6	136.66 \pm 5.7	54.9 \pm 2.1	0.436 \pm 0.02
	Percolation	Leaf	26.7	213.15 \pm 8.7	88.00 \pm 2.9	106.2 \pm 3.4	0.401 \pm 0.02
	Percolation	Flower	30.7	132.54 \pm 5.6	98.95 \pm 3.2	208.4 \pm 4.9	0.415 \pm 0.01
	Soxhlet	Leaf	20.8	688.97 \pm 23.3	121.19 \pm 5.4	24.3 \pm 0.9	0.262 \pm 0.00
	Soxhlet	Flower	28.1	321.38 \pm 13.2	121.50 \pm 4.8	61.1 \pm 1.4	0.237 \pm 0.01
<i>O. orientalis</i>	Ultrasonic	Aerial	14.1	361.55 \pm 12.3	46.81 \pm 1.7	17.9 \pm 0.8	0.634 \pm 0.02
	Percolation	Aerial	23.4	384.48 \pm 14.7	60.56 \pm 2.4	31.9 \pm 0.9	0.399 \pm 0.01
	Soxhlet	Aerial	18.5	323.28 \pm 12.0	62.42 \pm 2.3	43.3 \pm 1.8	0.479 \pm 0.01
<i>D. racemosa</i>	Ultrasonic	Leaf	8.5	248.28 \pm 10.1	136.25 \pm 3.4	135.9 \pm 4.9	0.283 \pm 0.00
	Percolation	Leaf	14.4	268.97 \pm 9.3	146.60 \pm 5.5	115.6 \pm 2.8	0.395 \pm 0.01
	Soxhlet	Leaf	20.1	256.06 \pm 11.5	131.17 \pm 4.0	104.6 \pm 2.6	0.236 \pm 0.01
<i>S. ebulus</i>	Ultrasonic	Flower	12.0	81.33 \pm 3.2	52.41 \pm 1.6	617.3 \pm 22.8	0.344 \pm 0.01
	Percolation	Flower	15.0	84.31 \pm 2.5	49.93 \pm 1.1	9.5 \pm 0.9	0.589 \pm 0.03
	Soxhlet	Flower	33.4	75.69 \pm 1.8	51.55 \pm 2.2	9.8 \pm 0.4	0.247 \pm 0.00
<i>E. amoenum</i>	Ultrasonic	Petals	22.3	75.83 \pm 2.8	39.88 \pm 1.4	151.9 \pm 3.6	0.222 \pm 0.01
	Percolation	Petals	29.0	74.21 \pm 2.1	42.64 \pm 1.6	162.3 \pm 4.1	0.124 \pm 0.00
	Soxhlet	Petals	31.0	84.25 \pm 3.4	37.93 \pm 1.5	99.6 \pm 4.5	0.208 \pm 0.01

Values are expressed as mean \pm SD of three determinations

The values of total phenolics and flavonoids content in the extracts are shown in Table 1. The highest value of phenolic compounds was that *A. julibrissin* leaf extract prepared by soxhlet extractor (688.97 ± 23.3 mg GAE/g of extract). *A. julibrissin* leaf extract prepared using ultra sonication also had high content of phenolic compounds as well (565.52 ± 22.8 mg GAE/g of extract). However, the highest flavonoids content (146.60 ± 5.5 mg QE/g of extract) was that of *D. racemosa* leaf extract prepared by percolation method. *A. julibrissin* leaf extract (prepared by ultrasonication, 139.66 ± 6.2 mg QE/g of extract) had considerable quantity of flavonoid compounds as well. For the SPF values of the extracts, the highest values were for *Cucumis melo* leaf extract (obtained by ultra sonication) and *Artemisia absinthium* shoots (methanolic extract) with SPF values of 0.841 and 0.717, respectively.

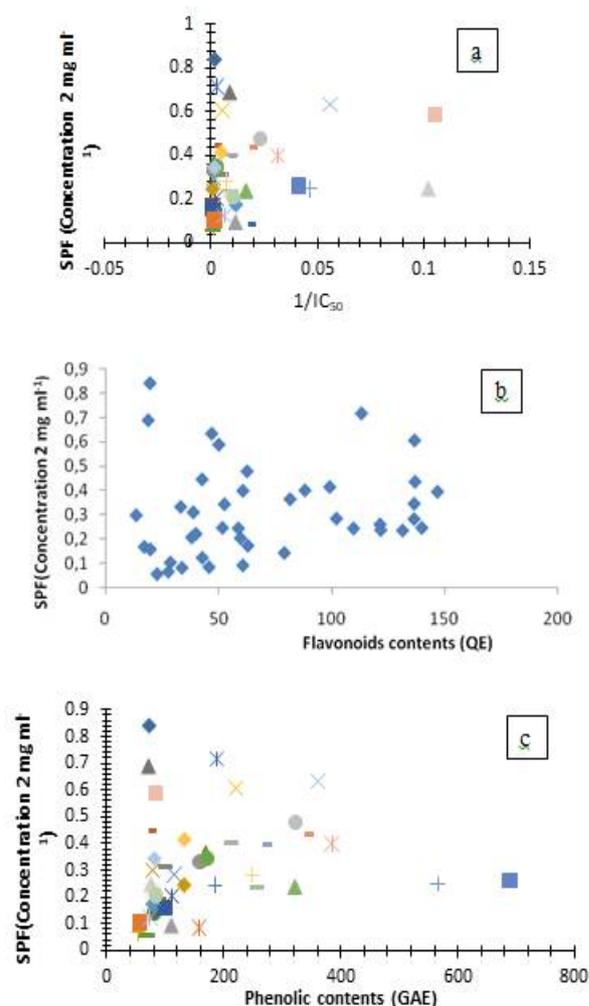


Figure 1: Correlation between SPF and antioxidant activity (a), flavonoid content (b) and phenolic content (c) in 42 tested extracts

The correlation between SPF with either phenolic ($r = 0.469$, $p = 0.003$) and flavonoid ($r = 0.355$, $p = 0.023$) contents and antioxidant activity was significant. But no significant correlations existed between SPF and antioxidant activity ($r = 0.242$, $p = 0.132$) in the extracts (Figure 1).

DISCUSSION

In this research, 42 extracts from nine known medicinal plants were evaluated for their SPF using UV spectrophotometry. We found that *S. ebulus* flower extract (obtained by percolation), *S. ebulus* flower extract (obtained by soxhlet extractor), and *O. orientalis* shoot extract (obtained by ultra-sonication) had considerable antioxidant activity when compared with vitamin C and butylated hydroxyl anisole (BHA).

Antioxidant activity is important in UV protection [22]. It seems that the already reported photoprotective effect of *p. umbellata* is due to its antioxidant activity [23]. The flavonoids and polyphenols studied are good candidates for use in photoprotective products [24,25]. Phenolic compounds are believed to be capable of acting in redox-sensitive signaling cascades to inhibit DNA damage [23,26]. They may also be beneficial in preventing UV-induced oxygen free radical generation and lipid peroxidation.

We found that *A. julibrissin* and *D. racemosa* leaf extracts have high quantities of phenolic compounds. The highest values were obtained by ultrasonic extract of *C. melo* leaf and percolation extract of *A. absinthium* aerial parts with SPF values of 0.841 and SPF = 0.717 respectively. High SPF values have been reported from leaf extract of *D. moldavica* and flowering tops of *V. tricolor* (24.79 and 25.69, respectively) [21] often considered to be due to high phenolic contents [20]. The protective power identified in this study is vital as UV is highly genotoxic agent whose deleterious effects on human skin at DNA level has been widely accepted [22]. To prevent UV-mediated DNA damage, sun protection factors are of highly interests. Although UV radiation has some benefits, e.g., for the biosynthesis of vitamin D, its negative impact on human health is much more. Skin cancer is one of the serious results.

CONCLUSION

There is a relatively significant correlation between SPF and both phenolic and flavonoid contents but not antioxidant activity. The application of ultrasonically assisted extract may be useful in achieving high SPF values in some plants.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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