Bulgarian Journal of Agricultural Science, 30 (No 4) 2024, 599–602

Evaluation of *Achillea millefolium* L. for the photoprotection potential by UV spectrophotometric methods

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Abstract

Berbatovci-Ukimeraj, M., Osmani, I., Haziri, A., Faiku, F., Daci, M., Nuro, A., Govori, A., Ibrahimi, H., Misini, M. & Qerimi, B. (2024). Evaluation of *Achillea millefolium* L. for the photoprotection potential by UV spectrophotometric methods. *Bulg. J. Agric. Sci., 30*(4), 599–602

In recent years, the requirements for the use of herbal products and formulations against the solar radiation have increased, trying to avoid the excesive use of chemicals considered harmful to human health. The aim of this study was to determine the value of the sun protection factor (SPF) from the plant extracts of *Achillea millefolium* L. using solvents of different polarity such as ethanol, ethyl acetate and hexane. Determination of the SPF value of plant extracts was carried out by UV-Vis spectrophotometer measurements in the region of ultraviolet radiation, in the range of wavelengths 290–320 nm. From the obtained results it has been observed that all the extracts show protective ability against ultraviolet radiation. The highest SPF value of 35.5 was obtained from *A. millefolium* L. extracts with ethyl acetate extracted by Soxhlet extraction. While *A. millefolium* L. extracts with hexane, extracted with cold extraction, showed the lowest SPF value of 8.06. From the results achieved, we have estimated that the plant *A. millefolium* L. has high photoprotective potential and as such its extracts can be used in sunscreen formulations. The spectrophotometric method used is fast, simple and cost-effective for the *in vitro* determination of SPF values.

Keywords: Achillea millefolium L.; sun protection factor (SPF); UV-VIS spectrophotometry

Introduction

Ultraviolet light is classified by the World Health Organization (WHO) as carcinogenic and produces several adverse effects including mutagenicity, skin immune depression, accelerated skin aging, and photodermatoses. Solar ultraviolet radiation (UVR) is divided into three categories: UVC (200–280 nm), UVB (280-320 nm) and UVA (320–400 nm) (Mbanga et al., 2015). The most biologically harmful UVC radiation is absorbed by the ozone layer, but UVB radiation can still pass through the ozone layer and is responsible for skin damage from sunburn. Furthermore, UVA radiation can reach the deeper layers of the epidermis and dermis, and the harmful effects provoke premature aging of the skin (Malsawmtluangi et al., 2013; More et al., 2013; Kale et al., 2011; Mansur et al., 1986).

In view of such circumstances, skin protection using creams with a high sun protection factor (SPF) can be essential for reducing the harmful effects of exposure to ultraviolet radiation. Therefore, concerted efforts in developing sunscreen formulations that effectively protect human skin against UVA- and UVB-induced damage are of scientific and commercial value (Dutra et al., 2004; Vettor et al., 2008).

Among the active compound sources that exhibit sun protective properties against UV-induced damage are those extracted from plants. It has been shown that plants produce

various antioxidants, as well as compounds that quench and protect against molecular damage caused by reactive oxygen species (ROS). The most important group of compounds includes flavonoids which are known for their ability to scavenge UV-induced radicals, a feature that also indicates that flavonoids make excellent shields for absorbing UV radiation (Solovchenko et al., 2003; Mukherjee et al., 2011).

Until now, only a limited number of plant extracts and oils exhibiting photoprotective action have been used in sunscreen formulations. Herbal sunscreens are safe, widely accepted by consumers, and also work in a variety of ways, playing multiple roles in ameliorating the carcinogenesis process. However, the current selection of plant extracts, containing sun protective ingredients for the preparation of sunscreens, are quite limited and insufficient to qualify the increased consumer requirements for cosmetics formulated using products obtained from natural sources (Mohamad et al., 2018).

A. millefolium L. is a widespread plant of the Asteraceae family restricted to the Northern Hemisphere. The genus name originates from its ancient use as a wound-healing medicine by the Trojan hero Achilles, while the word millefolium, meaning a thousand leaves, refers to the leaves (Benedek & Kopp, 2007). *A. millefolium* L. is one of the most popular medicinal plants, used for many centuries as a natural remedy for the treatment of wounds, hemorrhage, headaches, inflammation, pain and gastrointestinal disorders. The medicinal properties of *A. millefolium* L. extracts and compounds have also been confirmed by a wide range of scientific studies. *A. millefolium* L. extracts, juices, essential oil are also common and valuable active ingredients in cosmetics, acting as skin conditioners, antioxidants and cooling agents. Recent studies have proven the strong antioxidant effect, skin lightening, wound healing, skin rejuvenation and anti-inflammatory potential of alcoholic or hydro-alcoholic extracts of *A. millefolium* L. Cosmetic ingredients containing *A. millefolium* L. preparations are also considered as safe (Gaweł-Bęben et al., 2020).

A wide variety of bioactive components, including amino acids, fatty acids, salicylic and succinic acids, ascorbic acid, folic acid, caffeic acid and flavonoids are found in *A. millefolium* L. extracts. Among them, flavonoids (kaempferol, luteolin and apigenin) have attracted more attention in recent years and most of the antioxidant and anti-inflammatory properties of this plant are attributed to them (Ayoobi et al., 2017).

The aim of this study was to evaluate another potential source of the plant *A. millefolium* L. for the sunscreen compounds. *A. millefolium* L. extracts were evaluated for their photoprotective potential by using UV-VIS spectrophotometer.

Materials and Methods

Plant Material

The dried plant *A. millefolium* L. used in this research was purchased from the market. The plant is ground in a grinder, and then placed in a laboratory glass and sealed with cling film.

Preparation of Extracts

The dried powder of *A. millefolium* L. (10 g for each solvent) was extracted by the Soxhlet extraction within 4 h by using ethanol (A_{E1}), ethylacetate (A_{EA1}), hexane (A_{H1}), and in the cold by leaving plant in the mixer for 24 hours by using the same solvents; ethanol (A_{E2}), ethylacetate (A_{EA2}) and hexane (A_{H2}) . Extraction was performed with different solvents such as ethanol, ethyl acetate and hexane. The extracts were concentrated under reduced pressure using a rotary evaporator to dryness in which six different extracts (preparats) were obtained (A_{E1}), (A_{EA1}), (A_{H1}), (A_{E2}), (A_{EA2}), (A_{H2}).

Preparation of solutions for spectrophotometric measurement

To carry out the measurements in the UV-VIS spectrophotometer, solutions with a final concentration of 1 mg/mL were prepared from the dry extracts. The dry extracts were dissolved in the ethanol. The measurements were performed in a quartz cuvette with a thickness of 1 cm. The ethanol was used as blank, which were also used for the preparation of the solutions. We measured the absorbance for each solution and from the obtained data we built the spectra for each case. We used the obtained absorbance results to calculate the sun protection factor (SPF) by the equation developed by Mansur (Mansur et al., 1986).

$$\mathrm{SPF}spectrophotometric = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times A(\lambda),$$

where $EE(\lambda)$ defined as the erythemal spectrum effect, $I(\lambda)$ is the solar intensity spectrum, $A(\lambda)$ is the absorption of the sun protection product, CF is a corrective factor ($CF = 10$).

Result and Discussion

SPF is a quantitative measure of the effectiveness of a sunscreen formulation. To be effective in preventing sunburn and other skin damage, a sunscreen product must have a broad absorbance range between 290 nm and 400 nm (Mohamad et al., 2018). The potential of *A. millefolium*-ethanol (A_{E1}), *A. millefolium*-ethyl acetate (A_{EA1}) and *A. millefolium*-hexane (A_{H1}) extracts as sun protection agents was measured by UV absorption of solutions prepared with *A. millefolium* L. extracts in the UVB radiation range.

The absorbance of solutions of extracts of *A. millefolium* L. was measured with the UV-VIS spectrophotometer, in a quartz cuvette, with a thickness of 1 cm. The absorbance was measured in the region of wavelengths from 290 nm to 320 nm, in the interval of 5 nm. The ethanol solvent for each solution was used as a blank. The absorbance data from the measurements performed and the SPF values calculated with Mansur's equation are presented in Tables 1 and 2.

Table 1. Absorbance and SPF values of Soxhlet extracted products

Wavelength, nm	Soxhlet extraction		
	Ethanol	Ethyl acetate	Hexane
	A_{E1}	A_{AE1}	A_{H1}
290	3.2	3.828	1.4826
295	3.2	3.669	1.322
300	3.18	3.559	1.229
305	3.18	3.509	1.173
310	3.16	3.512	1.115
315	3.07	3.601	1.081
320	2.96	3.686	1.014
SPF	31.65	35.53	11.85

Table 2. Absorbance and SPF values of cold extracted products

Wavelength, nm	Cold extraction		
	Ethanol	Ethyl acetate	Hexane
	A_{E2}	A_{AE2}	A_{H2}
290	3.48	3.17	0.991
295	3.46	3.03	0.884
300	3.45	2.981	0.832
305	3.3	2.992	0.794
310	3.3	3.044	0.765
315	3.26	3.179	0.753
320	3.17	3.286	0.734
SPF	33.5	30.26	8.06

From Tables 1 and 2 it can be seen that the higher SPF values showed the extracts of ethyl acetate (35.53 and 30.26) and ethanol (31.65 and 33.5), this may be due to the presence of flavonoids, flavones, phenolic acids such as and other phytoconstituents in these extracts.

According to the guidelines of international regulatory agencies, only SPF value equal to or greater than 6 is suitable for use in cosmetic products (Costa et al., 2015). Therefore, the results suggest that *A. millefolium* L. extract can be considered as a promising active ingredient due to its high SPF value (35.53) at low concentration level (1 mg/mL). If higher SPF is required for the formulation, it can be achieved by reducing the dilution factor during the preparation of *A. millefolium* L. extract because SPF has been found to be concentration dependent (Costa et al., 2015). One of the most important factors affecting the efficiency of extracting bioactive compounds from plant materials is the extraction solvent (Van et al., 2017).

The best solubility of the photoprotective component in ethanol and ethyl acetate can be attributed to its chemical structure of flavonoids and alkaloids. The characteristic conjugated system in these phytochemicals enables them to absorb high intensity UV rays, thus producing better SPF (Saewan & Jimtaisong, 2013) Lower SPF values showed the hexane extracts (11.85, and 8.06).

During this research the absorbance of extracts were measured at wavelengths from 230 nm to 400 nm, in the interval of 2 nm, measured with a UV-VIS spectrophotometer in a 1 cm quartz cuvette. Based on these data, absorption spectra were constructed.

In the following figures (Figures 1 and 2), the spectra of the extracts in the region from 230 nm to 400 nm are constructed. Spectra were constructed for the extracts: *A. millefolium*-ethanol (A_E), *A. millefolium*-ethyl acetate (A_{EA}) and *A. millefolium*-hexane (A_H).

Figure 1. shows the spectra of extracts of *A. millefolium* L. with ethanol, ethyl acetate and hexane extracted by hot continuous-Soxhlet extraction.

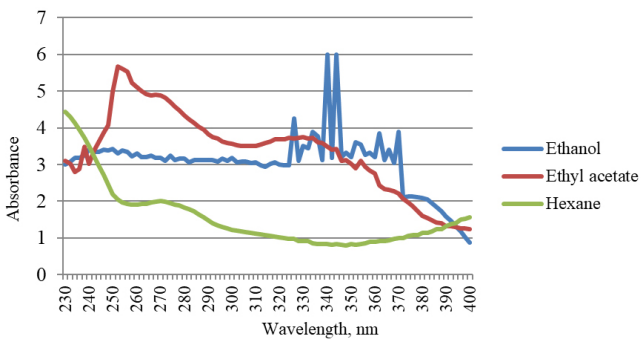

Fig. 1. UV spectra of extracts obtained by Soxhlet extraction

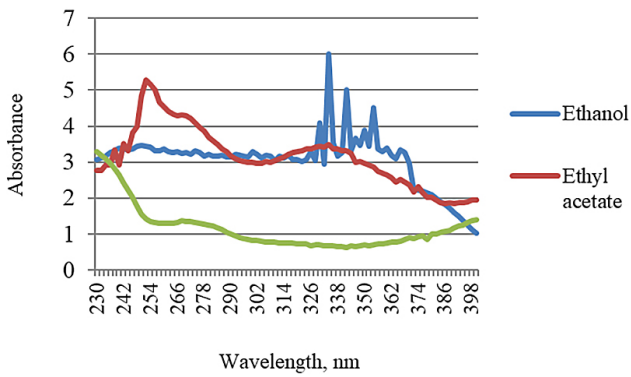

Fig. 2. UV spectra of cold extraction extracts

Figure 2 shows the UV spectra of extracts of *A. millefolium* L. with ethanol, ethyl acetate and hexane extracted by cold extraction.

From the presentation of the UV spectra of extracts of *A. millefolium* L. with the extraction solvents, it can be seen that depending on the solvent used, the region of wavelengths where the maximum absorbance is reached also changes. This difference is much smaller if comparing the spectra of the same solvent with different extraction methods, Soxhlet and cold extraction.

Conclusion

The spectrophotometric assay for the measurement of sun protection factor (SPF) is a simple, reliable, rapid, inexpensive and validated technique that has been widely used to determine the potential of several sunscreen formulations of natural and synthetic products.

From the obtained results we can conclud that extracts of *A. millefolium* L. with ethanol, ethyl acetate and hexane have photoprotective properties, suggesting that these solvents were efficient in extracting high concentrations of active photoprotective compounds. The high SPF values obtained for *A. millefolium*-ethyl acetate (35.53) and *A. millefolium*-ethanol (31.65) extracts, as well as the high absorption of UVA and UVB radiation, are good indications that these extracts will to be useful as protective photo-additives in sunscreen formulations.

Along with their many beneficial effects and safety, these botanical substances can become good, inexpensive and readily available ingredients for sunscreen formulations. Considering the favorable results of *A. millefolium*-ethyl acetate and *A. millefolium*-ethanol extracts, an *in vivo* experiments, to prove the efficacy of these extracts for application in sunscreen formulations, may be necessary.

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Received: **January, 11, 2023**; *Approved*: **August, 08, 2023;** *Published:* **August, 2024**