Research Article GU J Sci 36(1): 108-118 (2023) DOI: 10.35378/gujs.1052131

Gazi University

Journal of Science

http://dergipark.gov.tr/gujs

Highlighting the Cosmeceutical Potential of the Edible *Bunium alpinum* Waldst& Kit (Apiaceae) Growing in Algeria: *in vitro* Antioxidant and Photoprotective Effects

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Highlights

- This paper focuses on sun protection factor of plants, specially *Bunium alpinum* Waldst. & Kit.
- Apigenin-7-*O*-rutinoside is an important compound in the plant, and it was partially separated.
- It was found that both plant extracts and Apigenin-7-*O*-rutinoside were effective on sun protection.

Article Info

Received: 03 Jan 2022
Accepted: 10 Mar 2022

Keywords

Antioxidant activity
Bunium alpinum
Photoprotective activity
Sun protection factor
Ultraviolet visible- B

Abstract

The interest of using natural ingredients in cosmetic products has getting increased specially developing in new photoprotective formulations. In this study, it was undertaken to evaluate the cosmeceutical potential of the aerial parts of the species *Bunium alpinum* Waldst. & Kit (Apiaceae family) growing Algeria, by assessing their photoprotective and antioxidant effects. For this purpose, the total antioxidant capacity and activity were determined by using phosphomolybdenum and DPPH· radical scavenging methods. The *in vitro* photoprotective effect against UV-B radiations was determined according to the Sun Protection Factor (SPF) by using UV spectrophotometer at the range between 290 and 320 nm and Mansur equation. It was clear that among the tested extract, content of phenolic compounds and flavonoids of butanolic extract (Ba-BuOH) had higher than other extracts and it possessed strong antioxidant activity in DPPH· radical scavenging (20.65±0.017 mg VCE/g) and total antioxidant activity (196.69±4.08 mg VCE/g) assays. Similarly, its major compound Apigenin-7-*O*-rutinoside exhibited high photoprotective activity in UVB and UVA range and the SPF values were (14.70±0.11 and 32.80±0.5, respectively). The results gathered from our work reveal the using possibility of this extract and its major as antioxidant and sunscreening agent in cosmetic formulations.

1. INTRODUCTION

In the last few years, the use of natural ingredients in cosmetic products is the modern trend, thus medicinal and aromatic plants have gained a significant attention to be used as a natural source of antioxidants and solar filters in cosmetic preparations.

The dangerous effects of solar radiation on the skin are mostly occurred by the ultraviolet radiations (UVR) and the ongoing exposure to UVR can induce erythema, sunburns and premature aging of skin [1]. Thus the protection of the skin from UVR becomes necessary, and the use of preparations containing solar filters

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(UV absorbing agents) is important for the more effective prevention [2]. Indeed, it is well known that artificial UV filter agents have been largely utilized as photoprotective agents, but their protection has been widely debated because of their photo irritation and photosensitization [3]. Hence to reduce the harmful effects related to the synthetic UV filter; the use of the natural ingredients is evident since they are hypoallergenic [4]. The antioxidant potential of natural ingredients is one of the biological activities that has importance in cosmeceutical. Many studies have reported that antioxidants are useful to prevent the degradation of active ingredients in cosmeceutical products, and to defend the skin and slow up the skin aging procedure [5]. Therefore medicinal plant extracts have drawn the attention of cosmetic formulators to test their incorporation in cosmetic preparations owing to their phytochemical content and antioxidant potential [6].

Apiaceae family contains more than 300 genera and more than 3000 species over the world, several plant based therapeutic products from this botanic family are found to be used as antiseptic, carminative or vasodilator agents [7]. The genus *Bunium* L (Apiaceae) consists of about 100 species in the world and they are regularly cultivated in Algeria and other countries [8]. In Algeria, it is one of the most important aromatic and medicinal plants that involves seven species and four of them are endemic. Its different parts such as seeds and essential oils have been used in nutrition and medicine worldwide for so long [9]. Essential oil of *Bunium s*pecies had antimicrobial, anti-inflammatory, and antioxidant activities [10]. In this study, the preliminary phytochemical screening, total phenolics and flavonoids, *in-vitro* photoprotective and antioxidant activities of the extracts of the aerial parts of this plant were performed firstly to find out new non-chemical origin that could be used as sun screening agent and as a non-synthetic antioxidant in cosmetic products.

2. MATERIAL METHOD

2.1. Plant and Chemicals

Bunium alpinum Waldst. & Kitaerial parts were collected from Setif (eastern Algerian) in March 2015 (flowering stage) and was characterized by Prof. Dr. H. Laouer (Department of Biology and Plant Ecology, Ferhat Abbas University, Setif, Algeria). Voucher specimens were deposited in the Herbarium of our laboratory. All the chemicals were used as analytical grade. Thermo, Waltham, MO, USA branded UV/VIS spectrophotometer was used for the analyses.

2.2. Extraction, Apigenin-7-*O*-rutinoside Separation and Structure Elucidation

B. alpinum were dried at room conditions. About 300 g of dried aerial parts were firstly extracted with MeOH/CH_2Cl_2 (1:1) at 25 C° for 72 h. The macerate was concentered to dryness under vacuum at 40°C by a rotary evaporator to produce raw methanolic extract of *B. alpinum* (Ba-MeOH). The raw Ba-MeOH was mixed with water and kept in cold overnight then filtered, and the filtrate was extracted with n-butanol. The obtained butanolic extract of *B. alpium* (Ba-BuOH) was concentered to dryness under vacuum at 40°C by a rotary evaporator and kept at +4C° until the analyses. Ba-BuOH was utilized to column (MN SC6) and eluted with a gradient of toluene- MeOH (70-30%) with increased polarity. Fractions eluted with toluene-MeOH gave yellow precipitate, which was washed with MeOH to give Apigenin-7-*O*-rutinoside (105 mg). UV, 1H NMR and 13C NMR experiments were used to identify the structure of Apigenin-7-*O*-rutinoside and the spectroscopic properties were compared with literature data.

2.3. Preliminary Phytochemical Screening

The known amount of Ba-MeOH crude and Ba-BuOH fraction were resolved in methanol. Then these samples were used for detection of the poise of bioactive groups such as alkaloids, phenols, tannins, and flavonoids according to [11]. The final solution was followed for colour changing and/or precipitating forming for point out results.

2.4. Detection of Total Phenolic Substances (TPC)

The amount of total phenolic substances was determined by using Folin–Ciocalteu method [9]. Known amount of Ba-MeOH crude extract and Ba-BuOH fraction solution (1mg/mL in methanol) were used for determination. The optical density was read at 765 nm after 2h. The findings represented as gallic acid equivalents per dry weight (mg GAE /g DW).

2.5. Detection of Total Flavonoid Substance (TFC)

The amount of total flavonoid substance was carried out by using $AlCl_3$ (in ethanol) method [12]. Ba-MeOH crude extract and Ba-BuOH fraction solution (1mg/mL in methanol) were used for determination. The optical density was recorded at 430 nm. The findings were presented as quercetin equivalents (mg QE /g DW).

2.6. DPPH· Radical Scavenging Assay

The free radical scavenging activity was detected according to [13] with minor modifications. Known amount of Ba-MeOH crude extract, Ba-BuOH fraction and Apigenin-7-*O*-rutinoside or standard (BHT) were incubated for reaction with DPPH· radical solution (0.004% prepared in ethanol) for 30 minutes in dark and optical density was recorded at 517 nm. The findings were represented as vitamin C equivalents mg VCE /g.

2.7. Phosphomolybdenum Test for Total Antioxidant Capacity

The total antioxidant capacity (TAC) of Ba-MeOH crude extract, Ba-BuOH fraction and Apigenin-7-*O*-rutinoside was measured by the phosphomolybdenum method according to [14] with minor modifications. The optical density was read at 695 nm. The results represented as vitamin C equivalents mg VCE/g.

2.8. UV-A and UV-B Absorbing Potential

The UV filtering potential of Ba-MeOH crude extract, Ba-BuOH fraction and Apigenin-7-*O*-rutinosidewas performed described by [15] with minor modifications. The UV absorption of Isoorientin (200 µg/mL) was recorded between 290-400 nm. The area under the curve (AUC) was calculated from the integral of the absorbance between 290-400 nm.

2.9. Detection of *In vitro* Sun Protection Factor

Currently, several cosmetic laboratories use two distinguish *in vitro* methods to carry out the sun protection factor (SPF). The first procedure consists to assess the absorption or transmission of UV radiations via a film of sunscreen applied to suitable substrate such as 3M Transpore tapes and polymethyl methacrylate (PMMA) plates, while in the second method the characteristics of absorption the sunscreen agents were found out by spectrophotometric method [16].

The determination of *in vitro* SPF of Ba-MeOH crude extract, Ba-BuOH fraction and Apigenin-7-*O*-rutinoside were performed by the dilution method described by [17, 18]. For this, 10 mg of sample was weighed and diluted in ethanol (0.2 mg/mL) seperately and sonificated for 5 minutes. Subsequently, spectrophotometric readings at wavelengths 290 to 400 nm with intervals of 5 nm were determined.
The SPF between 290nm and 320 nm was calculated using Mansur equation [19].

2.10. Statistical Calculations

All analyses were done in triplicate and the data points are presented as Mean ± SD. Statistical calculations were carried out using Graph pad prism 7.04 and the statistical significance of the differences were determined by t-test and one-way analysis of variance (ANOVA) at a confidence level of 95% ($p_a = 0.05$) followed by the Post Hoc Tukey test ($p_a = 0.05$).

3. THE RESEARCH FINDINGS AND DISCUSSION

$UV_{\lambda max}$ (MeOH) of Apigenin-7-*O*-rutinoside (Figure 1) was found between 268 and 330nm. 1H-NMR (250 MHz, DMSO-d6, δ in ppm) data: 6,78 (1H, d, J=1,5 Hz, 8-H), 6,46 (1H, d, J=1,5 Hz, 6-H), 6,87 (1H, s, 3-H), 6,95 (2H, d, J=8,6 Hz, '3-H / '5-H), 7,95 (2H, d, J=8,6 Hz, '2-H / '6-H), 5,06 (1H, d, J=6.8 Hz, 1''-H), 5,3 (1H, br, 1'''-H), 1,06 (3H, d, J=6.05 Hz, CH_3)

13C-NMR (250 MHz, DMSO-d6, δ in ppm) data: 164,5 (C-2), 103,1 (C-3), 182,1 (C-4), 161,3 (C-5), 99,6 (C-6), 163 (C-7), 94,9 (C-8), 157 (C-9), 105,5 (C-10), 121,1 (C-1'), 128,8 (C-2', C-6'), 116,2 (C-3', C-5'), 161,4 (C-4'), 99,9 (C-1''), 73,2 (C-2''), 76,3 (C-3''), 69.6 (C-4''), 75,7 (C-5''), 66,1 (C-6''), 100,6 (C-1'''), 70,3 (C-2'''), 70,7 (C-3'''), 72 (C-4'''), 68,4 (C-5'''), 17,9 (C-6'''). It was clear that the 1H-NMR and 13CNMRdata are compatible with those of Apigenin-7-*O*-rutinoside in the literature [20, 21].

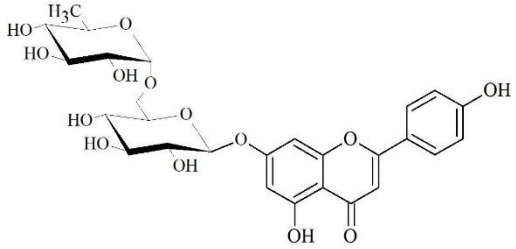

Figure 1. *Apigenin-7-O-rutinoside isolated from B. alpinum aerial parts*

From the preliminary phytochemical screening test results of Ba-MeOH raw extract and Ba-BuOH fraction of *B. alpinum* aerial parts, it contains secondary metabolites compounds including polyphenols, flavonoids and tannins; findings are presented in Table 1.

Table 1. *Preliminary Phytochemical Screening of Ba-MeOH raw extract Ba-BuOH fraction*

Phytoconstituents	**Ba-MeOH**	**Ba-BuOH**
Alkaloids	-	-
Polyphenols	+	+
Flavonoids	+	+
Tannins	+	+

Symbol (+) indicates presence respective phytochemical, Symbol (-) indicates absence respective phytochemical. The results were detected by color change and/or precipitate formation

The presence of phytoconstituents in herbal extracts such as flavonoids has cytotoxic activity and protection effects on gastrointestinal infections, while tannin compounds are described to be able to inhibit HIV reproduction [22]. It is well mentioned that phenols and flavonoids in herbal extracts are water-soluble antioxidants, which behave as free radical scavengers and may contribute straight forwardly to the antioxidant effect and prevent oxidative cell damage, in addition the presence of these phytoconstituents and tannins can promote the capability of plant extracts to absorb UVR radiations, this could be explained the possibility to be used as photoprotective ingredients [23].

Consequently, the presence of these phytoconstituents in *B. alpinum* extracts could clarify the extensively medical uses of the plants belonging to the *Bunium* genus. The results of the total phenolic contents (Figure 2) of the two extracts of *B. alpinum* demonstrated that the Ba-BuOH fraction possessed the highest amount value of (190.27±0.74 mg GAE /g DW), in comparison with Ba-MeOH crude extract (35.51±0.15mg AGE /g DW). Also, Ba-BuOH fraction (85.66±2.76 mg QE/g DW) was greater than that of Ba-MeOH crude extract (14.50±0.25mg QE/g DW) according to total flavonoid content. According to the results of the

phenolics and flavonoids of methanolic raw extract of *B. alpinum* (Ba-MeOH), the findings are in accordance with [24]. They reported that methanolic extracts of *B. pinnatifolium* and *B. sayai* growing in Turkey, were found to contain amount of phenolics and flavonoids with (35.94±1.89mg AGE /g DW, 13.95±0.15mg QE/g DW), which is quite similar to that obtained by our research group. Concerning Ba-BuOH fraction, no data have been reported yet in term of their phenolics and flavonoids. Thus the presence of the higher levels of these phytoconstituents in the Ba-BuOH fraction may contribute to its pharmacological effects, particularly the antioxidant activity, this correlates well with previous reports of [25-27] who mentioned that the higher phenolic and flavonoid substances in plant extracts entail their significant antioxidant activities.

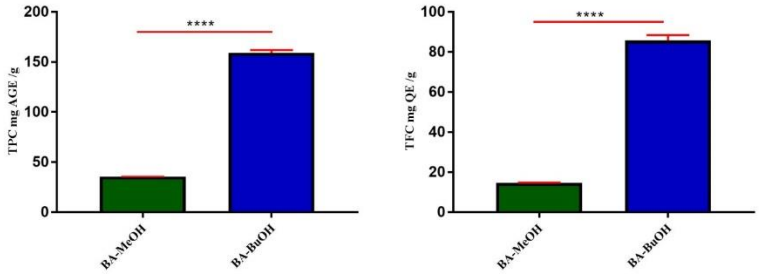

Figure 2. *Total phenolic (TPC) and flavonoid (TFC) substances of B. alpinum. **** $p < 0.0001$*

In this study, antioxidant activity was performed by two different, well established methods, namely, DPPH· radical scavenging and total antioxidant activities (TAC) (phosphomolybdenum test) (Figure 3). Indeed, the Ba-BuOH fraction was more potent as antioxidant agent than the Ba-MeOH crude extract. Noteworthy, the higher total phenolic and flavonoid substances in the Ba-BuOH fraction entails its greater antioxidant effects. Hence, there is a good relation between total phenolics, flavonoids and the antioxidant effect.

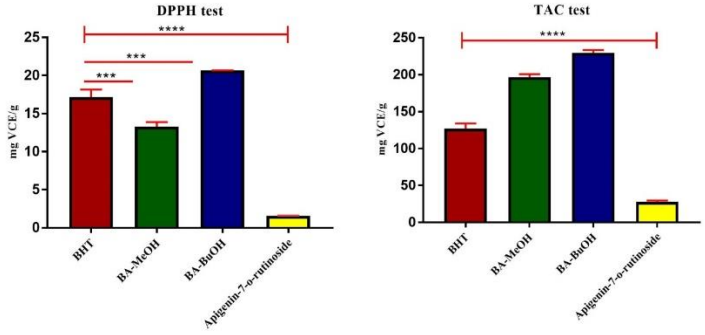

Figure 3. *Antioxidant activity of B. alpinum. $^{(***)}$ $p < 0.001$ between samples, $^{(****)}$ $p < 0.0001$*

In DPPH· radical scavenging activity, the findings showed that the most potent DPPH· radical scavenger was the Ba-BuOH fraction (20.65±0.017 mg VCE/g) followed by Ba-MeOH crude extract (13.30±0.58 mg VCE/g) while the weakest DPPH· radical scavenger was Apigenin-7-*O*-rutinoside (1.151±1.1009 mg VCE/g).

Similarly, in phosphomolybdenum test, the Ba-BuOH fraction recorded the strongest total antioxidant capacity (229.77±3.59 mg VCE/g) followed by Ba-MeOH crude extract (196.70±4.08 mg VCE/g), however, the weakest total antioxidant capacity was recorded by Apigenin-7-*O*-rutinoside (27.62±2.01 mg VCE/g). Interestingly, the Ba-BuOH fraction was more effective than the standard BHT in both DPPH· radical scavenging ($p<0.001$) and total antioxidant capacity ($p<0.0001$) assays (17.151±1.009, 127.039±7.034 mg VCE/g, respectively).

Antioxidant activity of herbal extracts is generally correlated with amount of phenolic and flavonoid substances, which may act antioxidant effects as free radical scavengers [28]. Thus, the significant DPPH· radical scavenging effect of Ba-BuOH fraction could be ascribed to the highest concentration in terms of phenolics and flavonoids present in this extract. These results are supported by previous reports on other Apiaceae species [24, 29, 30].

By assessing the results of total antioxidant capacity, it was noticed that the Ba-BuOH fraction possess strong antioxidant capacity compared to the Ba-MeOH, this could be explained by the presence of highest concentration of phenols and flavonoids. These compatibles well with previous studies that proved close correlation between the total antioxidant activity (TAC) of extracts and their phenolic and flavonoid substances [20].

In recent years the novel trend in cosmetic industries is the use of antioxidant molecules, especially those derived from natural origin, as effective alternatives to synthetic additives such as preservatives [31]. Therefore several studies suggested that since they defend skin cells from radical oxygen species (ROS), the association of natural antioxidants in dermo-cosmetic preparations is highly important [32]. Taking into account, these literature data and the findings for antioxidant effect recorded by our team, it seems that the Ba-BuOH extract of the species *B. alpinum* growing in eastern Algeria might be a promising antioxidant applicant to be incorporated into several dermo-cosmetic formulations.

UV spectrophotometric study was performed to assess the UV filtering potential of the two extracts and Apigenin-7-*O*-rutinoside (Figure 4, Tables 2 and 3). Actually, the results demonstrated that both extracts and Apigenin-7-*O*-rutinoside recorded UV absorbance in the range UVB 290 to 320 nm and UV-A (320-400 nm) radiations. Apigenin-7-*O*-rutinoside showed the highest UV-A and UV-B absorbing potential (AUA= 196.30±0.02) followed by Ba-BuOH fraction (AUC= 137.40±0.02), while Ba-MeOH crude extract was the less effective UV-A and UV-B absorbing agent (AUA= 37.30±0.03).

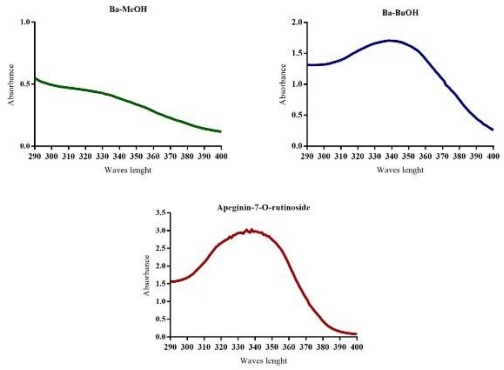

Figure 4. *UV-absorbing profile of B. alpinum*

The findings of the *in vitro* SPF were presented in Table 2. Actually, the results revealed that Apigenin-7-*O*-rutinoside recorded the highest SPF (32.80±0.5) followed by Ba-BuOH fraction (14.70±0.11). In contrast, the lowest value was registered by Ba-MeOH crude extract (8.20±0.22).

Table 2. *Normalized product functions for the calculation of SPF*

λ (nm)	**EE x I (Normalized)**
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839

320	0.0180
Total	1

EE: Erythemal effect spectrum; I: Solar intensity spectrum

By assessing the *in vitro* SPF value, Apigenin-7-*O*-rutinoside possessed the highest photoprotective potential. This is related with a previous report that demonstrated the significant photoprotective effect of flavonoids in UV-B and UV-A range [1]. The protective factor of Ba-BuOH fraction (SPF=14.70±0.11) is higher than that of Ba-MeOH crude extract (8.20±0.22), indeed the higher SPF (290-320) value of Ba-BuOH is probably linked to its phenol and flavonoid contents, which is correlate well with several authors who demonstrated that the photoprotective effect is mainly associated with the levels of phenolics and flavonoids in plant extracts [33- 38].

Table 3. *In vitro sun protection factors of Ba-MeOH crude extract and Ba-BuOH fraction and Apigenin-7-O-rutinoside*

Samples	**SPF (290-320)**	**AUC**
Ba-MeOH	8.20±0.22	37.30±0.03
Ba-BuOH	14.70±0.11	137.40±0.02
Apigenin-7-*O*-rutinoside	32.80±0.5	196.30±0.02

$p < 0.05$, AUC: area under curve

It is well reported that according to ANVISA (National Health Surveillance Agency of Brazil), the minimum value of SPF for photoprotective products is 6 [39], however the FDA (Food and Drug Administration of the United States) recommended that only SPF values that are greater than or equal to 15 are suitable for use in preparations with photoprotective effect [23]. Based on these recommendations, the results of SPF recorded in the current study revealed a satisfactory photoprotective effect for all tested samples, especially for Ba-BuOH extract and Apigenin-7-*O*-rutinoside. On the other hand, the concentration of Ba-MeOH crude extract, Ba-BuOH fraction and Apigenin-7-*O*-rutinoside in our study was 0.2mg/mL (0.02% w/w), indeed this concentration was found to be is much lower than the normal use dose of sunscreening agent in the cosmetic products such as benzophenone-3 could be used up to 5–6% w/w, sulisobenzone up to 5-10% w/w [40]. Consequently, our data suggest that Ba-BuOH and its pure main compound (Apigenin-7-*O*-rutinoside) could be useful ingredients for UV protective cosmetic formulations and preparations since they presented high sun protection factors at low dilution.

4. RESULTS

In summary, the current study estimate, total phenolic and flavonoid substances, *in-vitro* antioxidant as well as photoprotective activities aerial parts of *Bunium alpinum* Waldst. & Kit (Apiaceae) belonging to Algerian flora were performed firstly. The findings presented in this study indicated that the Butanolic extract was had higher phenolic and flavonoid substances and antioxidant activity. Furthermore, the obtained data showed that this extract and its main compound (Apigenin-7-*O*-rutinoside) exhibited high photoprotective activity and also it appears to be good candidates to be developed as sunscreening agents in cosmetic preparations and as a source of natural antioxidants.

CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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