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## Research Article

### ***Centaurea dimorpha* Viv. (Asteraceae) growing in Algeria extracts as a promising natural cosmetic active ingredient: Broad-spectrum photoprotection and antioxidant efficacy**

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#### Abstract

Recently, plants have been considered as a valuable natural source of cosmetic active ingredients owing to their sustainability and weak toxicity. Thus, this study was conducted to investigate the cosmetic efficacy of *Centaurea dimorpha* Viv (Asteraceae), an endemic species of North Africa, as a promising natural cosmetic active ingredient. In the present study, Ethyl acetate and Butanolic extracts obtained from powdered *Centaurea dimorpha* aerial parts were investigated for their phenolic and flavonoid contents, which were evaluated via Folin-ciocalteu reagent and aluminium chloride methods, the in vitro antioxidant potential was evaluated by DPPH radical scavenging, phosphomolybdenum and phenanthroline assays, the UVB and broad spectrum protective efficacy was spectrophotometrically assessed by measuring SPF, UVA/UVB ratio and critical wavelength (CW) indices. The highest levels of TPC and TFC were recorded by the ethyl-acetate extract ( $119.20 \pm 0.32 \mu\text{g GAE mg}^{-1}$ ,  $50.65 \pm 0.43 \mu\text{g QEmg}^{-1}$ , respectively). Similarly, this extract displayed a significant antioxidant effect, particularly in the phenanthroline assay ( $152.63 \pm 0.49 \mu\text{g AAEmg}^{-1}$ ). Ethyl acetate extract also showed UVB and broad-spectrum (UVB-UVA) protective efficacy (SPF= $12.30 \pm 0.001$ , UVA/UVB ratio= $0.63 \pm 0.001$ ,  $\lambda_c= 371$ ). The results obtained show the possibility to use Ethyl acetate extract as a promising active ingredient for sunscreen formulations.

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## 1. Introduction

Ultra Violet radiation from the sun, especially UVA (320-400 nm) and UVB (290-320 nm), causes DNA damage, which activates inflammatory processes and skin cancer (Ganesan & Hanawalt, 2016; Schuch et al., 2017). Protecting the human skin from these radiations is therefore, an essential strategy to be considered. In this context, the use of sunscreens is considered as one of the most widely used techniques to ensure photoprotection (Guan et al., 2021). Sunscreen formulations contain active ingredients that function as UV filters (Jansen et al., 2013). Despite their efficacy in protecting human skin, several studies reported that commercial sunscreen active chemicals may be hazardous to humans (Downs et al., 2016), which has raised interest in employing natural ingredients to design sunscreen formulations (Cefali et al., 2016; Serafini et al., 2015).

One of the biological properties of interest in cosmeceuticals is the antioxidant capacity of natural substances. Numerous scientists noted that anti-oxidants are helpful in preserving cosmetic active ingredients from degradation, and protecting against skin damage (Lohani et al., 2019). Therefore, plant extracts which are rich in anti-oxidant molecules have attracted the interest of cosmetic designers to study their incorporation into cosmetic products (Thibane et al., 2019). In addition to their significance as a potential source of cosmetic ingredients, plant extracts are also considered to be an important reservoir for drug development. Actually, Digoxin extracted from the plant *Digitalis lanata*, has been used in heart diseases (Kumar, 2016). Terpenoid compounds have been shown to possess anti-microbial and anti-viral effects, glycyrrhizin found in the roots of the specie *Glycyrrhiza glabra* is considered as an important antiviral compound (Adeosun & Loots, 2024). The genus *Centaurea* of Asteraceae family contains about 700 species of biennial and perennial plants rarely dwarf shrubs (Bancheva et al., 2022). This genus is divided into three subgenera, *Centaurea sensu stricto*, *Lopholoma* (Cass.) Dobroc. and *Cyanus* (Mill.) Hayek, the *Centaurea* species are widely present in different open habitats varying in soil type, from inland sand dunes to meadows and from acidic to calcareous grasslands (Wala et al., 2021). Some species such as *Centaurea raphanina* spp. *mixta* are cultivated because of their commercial benefits and nutritional value, indeed a recent study showed that the cultivated species conserved their proprieties regarding the nutritional value and the quality when compared to the wild one (Petropoulos et al., 2020). The *Centaurea* taxons are frequently disturbed in Mediterranean area and in Western Asia (Belkassam et al., 2019; Yayli et al., 2005; Ertas et al., 2014).

In the Algerian flora this genus contains about 45 species (Azzouzi et al., 2016). Traditionally, various *Centaurea* have been used as a source of herbal medicines to treat gynecological, digestive, and dermatological problems as well as kidney failure and urinary tract irritation (Reyhan et al., 2004). On the other hand, multiple folk medicinal applications of *Centaurea* species were also cited such as: diuretic, anti-febrile, and anti-malarial (Alamri et al., 2023). Currently, extracts and essential oils of several *Centaurea* species have been reported to have anti-cancer, anti-diabetic, anti-oxidant and antibacterial effects (Fattaheian-Dehkordi et al., 2021; Khammar & Djeddi, 2012). Besides their therapeutic uses, *Centaurea* species are also employed in gastronomy as edible flowers, in cosmetics and as a source of colour in industry (Pires et al., 2018). Polyphenols (flavonoids, lignans, and other phenolic compounds) and sesquiterpene lactone are the two most significant classes of active substances present in *Centaurea* plants (Aktumsek et al., 2011, 2013; Dhouibi et al., 2020). It has been published that sesquiterpene lactones detected in *Centaurea* possess anti-cancer, anti-inflammatory, and anti-microbial effects (Kebbi et al., 2021).

Considering the published data on the phytochemical composition of *Centaurea* species and their richness in polyphenols on the one hand, and the use of some them in the cosmetic field on the other hand, the present study aims at evaluating the aerial part extracts of *Centaurea dimorpha* Viv (endemic in North Africa) as a new natural cosmetic active ingredient for sunscreen cosmetic preparations by assessing their polyphenolic composition, antioxidant, UVB and broad-spectrum photoprotective efficacy.

## 2. Materials and methods

### 2.1 Plant Material

*C. dimorpha* aerial parts were collected from M'Sila (eastern of Algeria) in May 2018, and the plant was identified by Prof T. Hamel botanist at Annaba University.

### 2.2 Extracts preparation

*C. dimorpha* aerial parts were dried in the shade under the condition of a room temperature. An amount of 900 g of dried aerial parts were extracted with boiling 70% methanol at room temperature for 72 h. After filtration, the hydroalcoholic solution was removed using a rotary evaporator under vacuum at 40°C to obtain CD-MeOH extract. Then, it was suspended in hot water and kept in the cold overnight, then successively fractionated with Ethyl acetate and Butanol to obtain the CD-EtOAc (6g) and CD-BuOH (18.6g) extract, which were kept at 4°C until use.

#### 2.2.1 Phytochemical characterization of plant material

TPC was determined using Folin-Ciocalteu reagent (FCR) as described by (Boulacel et al., 2019). TPC was calculated from Gallic acid calibration curve ( $y = 0.01435x + 0.2567$ ;  $R^2 = 0.993$ ; 6.25-200  $\mu\text{g mL}^{-1}$ ), and estimated as  $\mu\text{g GAE mg}^{-1}$ . For the extracts or Gallic acid standard measurement, 300  $\mu\text{L}$  of sample was mixed with 1500  $\mu\text{L}$  of FCR (diluted 10-fold). After 4 min, 1200  $\mu\text{L}$  of 7.5%  $\text{Na}_2\text{CO}_3$  was added, and the solution was kept in dark for 2 h. The absorbance of the obtained mixture was recorded at 765 nm.

TFC was assessed according to the procedure proposed by (Ayad et al., 2018). TFC was calculated from quercetin calibration curve ( $y = 0.02554x + 0.0078$ ;  $R^2 = 0.997$ ; 2.5-40  $\mu\text{g mL}^{-1}$ ), and estimated as  $\mu\text{g QE mg}^{-1}$ . For the extracts or quercetin standard measurement, 1 mL of 2%  $\text{AlCl}_3$  (prepared in ethanol) was mixed with 1 mL of standard or sample. After, incubation for 10 min in darkness, the absorbance was measured at 415 nm.

### 2.3 Evaluation of the antioxidant efficacy

The antioxidant efficacy of the extracts was determined through three *invitro* spectrophotometric methods: the DPPH assay, as described by (Almeida et al., 2011); the phenanthroline test, according to (Szydłowska-Czerniak et al., 2008) procedure; and the phosphomolybdenum assay, as described by (Cherfia et al., 2020). These methods were previously used to analyse some plants growing in Algeria (Lefahal et al., 2023). Concisely, the antioxidant activity was determined from ascorbic acid calibration curve ( $y = -0.03461x + 0.8852$ ,  $R^2 = 0.996$ ; 12.5-20  $\mu\text{g mL}^{-1}$ ; at 517 nm); ( $y = 0.007221x + 0.2383$ ,  $R^2 = 0.99$ ; 6.25-50  $\mu\text{g mL}^{-1}$ ; at 510 nm) and ( $y = 0.00632x - 0.00022$ ,  $R^2 = 0.9902$ ; 12.5-500  $\mu\text{g mL}^{-1}$ ; at 695 nm) for DPPH, phenanthroline and phosphomolybdenum assays, respectively. The antioxidant efficacy was estimated as  $\mu\text{g AAEmg}^{-1}$ .

### 2.4 Photoprotection capacity of plant material

#### 2.4.1 UV absorption profile

To evaluate the UV absorption potential of *C. dimorpha* extracts, a spectral scan (290-400 nm) was performed as described by (Ayad et al., 2022). Briefly, sample solutions of 0.1  $\text{mg mL}^{-1}$  (in ethanol) were scanned between 290-400 nm, using a UV/VIS spectrophotometer.

#### 2.4.2 In vitro sun protection factor (SPF) measurement

The extracts and standard (benzophenone 3) were prepared at a concentration of 0.1 mgmL<sup>-1</sup> in absolute ethanol. The solution's absorbance was measured in the range (290-320 nm) every 5nm, using a UV/VIS spectrophotometer. The SPF was calculated as described by (Mansur et al., 1986), by using the following formula:

$$\text{SPF in vitro} = \text{CF} \sum_{290}^{320} \text{EE}(\lambda) \cdot \text{I}(\lambda) \cdot \text{A}(\lambda)$$

CF: correction factor; EE(λ): erythemal effect spectrum; I (λ): solar intensity spectrum were constant (Sayre et al., 1979). A(λ): absorbance of the sample.

#### 2.4.3 Broad spectrum protection evaluation

The broad-spectrum protection against UV radiation of the extracts was evaluated by calculating the critical wavelength (CW) and the ratio  $\frac{\text{UVA}}{\text{UVB}}$  parameters.

##### Critical wavelength (CW) determination

CW is determined following the formula developed by (Diffey, 2007).

$$\int_{290}^{\lambda_c} \text{A}(\lambda) d(\lambda) = 0.9 \int_{290}^{400} \text{A}(\lambda) d(\lambda)$$

A (λ): absorbance at wavelength λ.

The broadspectrum protection against UV radiation is considered when  $\lambda_c \geq 370$  nm (Caballero-Gallardo et al., 2022).

#### 2.4.4 Ratio UVA / UVB determination

The ration  $\frac{\text{UVA}}{\text{UVB}}$  is another parameter used for the assessment of the broad-spectrum photo-protection (Ferrero et al., 2010; Kostyuk et al., 2018), and it is calculated as follows:

$$\frac{\text{UVA}}{\text{UVB}} = \left[ \int_{320}^{400} \text{A}(\lambda) d(\lambda) / \int_{320}^{400} d(\lambda) \right] / \left[ \int_{290}^{320} \text{A}(\lambda) d(\lambda) / \int_{290}^{320} d(\lambda) \right]$$

Based on the star rating system, the UVA protection is considered: good (\*\*), superior (\*\*\*), and  $\geq 0.8$ : maximum (\*\*\*\*) when UVA/UVB ratio  $\geq 0.4$ , 0.6 and 0.8, respectively.

#### Statistical analysis

All measurements were done thrice. The results are expressed as means  $\pm$  SD, and statistically analysed by the T-Test by using Graphpad prism software. The differences between samples were considered significant when  $p < 0.05$ .

### 3. Result and discussion

#### 3.1 Phytochemical characterization

The outcomes of TPC and TFC are shown in Table 1.

Table 1. The phytochemical contents of CD-EtOAc and CD-BuOH extracts

Samples	TPC ( $\mu\text{g GAEmg}^{-1}$ )	TFC ( $\mu\text{g QE mg}^{-1}$ )
CD-BuOH	$79.37 \pm 1.13^{***}$	$28.24 \pm 0.30^{***}$
CD-EtOAc	$119.53 \pm 0.32^{***}$	$50.65 \pm 0.43^{***}$

(\*\*\*): Significant difference at  $p < 0.001$ .

By analysing the results of the phytochemical composition, it is shown that CD-EtOAc extract contains the highest content of phenolic compounds ( $119.20 \pm 0.32 \mu\text{g AGE mg}^{-1}$ ) compared to CD-BuOH extract ( $79.37 \pm 1.13 \mu\text{g AGE mg}^{-1}$ ). Likewise, for the TFC, CD-EtOAc extract contains a substantial amount of flavonoids ( $50.65 \pm 0.43 \mu\text{g QE mg}^{-1}$ ). However, the CD-BuOH extract had weak amount of flavonoids ( $28.24 \pm 0.30 \mu\text{g QE mg}^{-1}$ ).

When comparing the outcomes of the current study with those of other works conducted on *Centaurea* species, it is well showed that our data are in agreement with those of numerous scientists who reported the richness of the *Centaurea* species in polyphenols (Salachna et al., 2021; Özcan et al., 2019). Azzouzi et al. (2016) indicated that TPC and TFC amount in EtOAc extract of *Centaurea choulittiana* Pomel was higher than that present in BuOH extract, which is in line with our results. Similarly, Lahneche and collaborators (Lahneche et al., 2019), noted that TPC and TFC levels in EtOAc extract of *Centaurea sphaerocephala* L were significant than those detected in BuOH extract, but their values are higher than those reported for the *Centaurea dimorpha*.

### 3.2 Antioxidant activity evaluation

The antioxidant efficacy of *C. dimorpha* species was estimated with three *in vitro* antioxidant methods (DPPH, phenanthroline and phosphomolybdenum) and the results are shown in Figure 1.

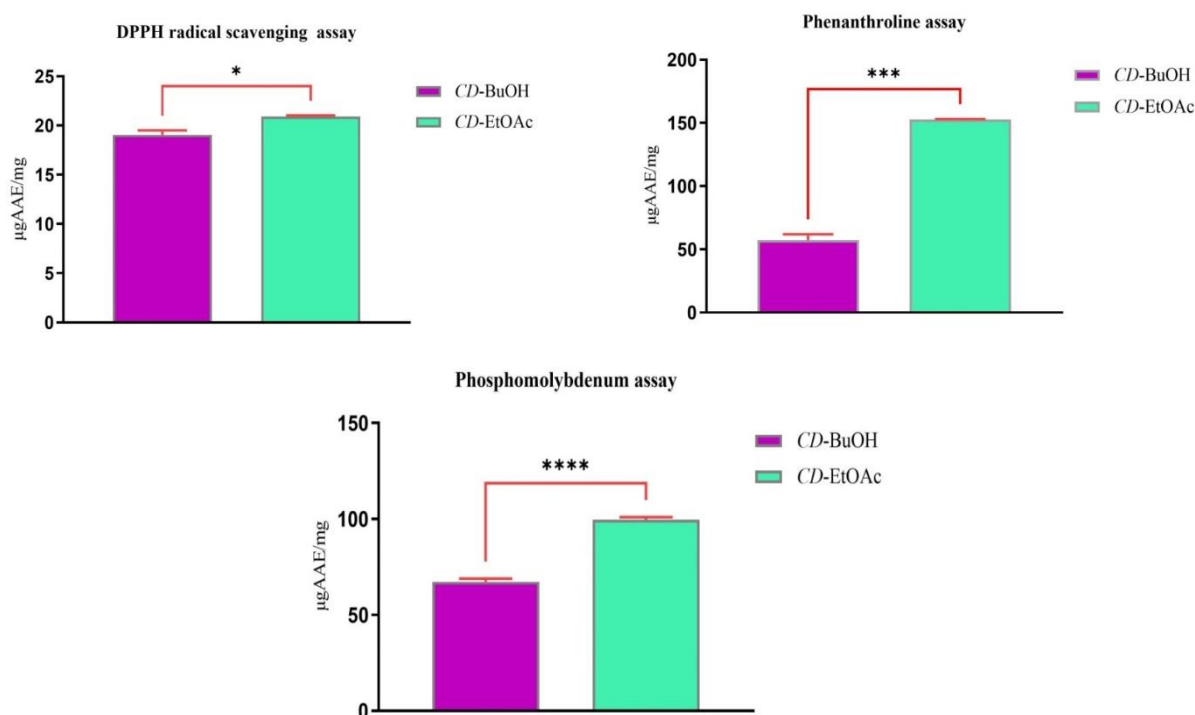


Figure 1. Antioxidant effect of *C. dimorpha* extracts. (\*\*\*\*), (\*\*\*) and (\*): Significant difference at  $p < 0.0001$ ,  $< 0.001$  and  $< 0.05$ , respectively

When shed light on the outcomes of the DPPH test, it is clearly observed that the *CD*-EtOAc extract had a good DPPH radical scavenging effect ( $20.91 \pm 0.13 \mu\text{g AAEmg}^{-1}$ ) compared to *CD*-BuOH extract. As for the obtained data of phosphomolybdenum assay (total antioxidant activity), it was also observed that *CD*-EtOAc extract demonstrated substantial efficacy ( $99.67 \pm 1.28 \mu\text{g AAEmg}^{-1}$ ), whereas the *CD*-BuOH extract was less effective ( $67.28 \pm 1.64 \mu\text{g AAEmg}^{-1}$ ). By assessing the results of phenanthroline assay, the results that *CD*-EtOAc extract was found to be more potent with ( $152.63 \pm 0.49 \mu\text{g AAEmg}^{-1}$ ) compared to *CD*-BuOH extract ( $57.19 \pm 4.72 \mu\text{g AAEmg}^{-1}$ ). By assessing the results of phenanthroline assay, it was found that the *CD*-EtOAc extract was more potent with ( $152.63 \pm 0.49 \mu\text{g AAEmg}^{-1}$ ) compared to the *CD*-BuOH extract ( $57.19 \pm 4.72 \mu\text{g AAEmg}^{-1}$ ).

While analysing the results of antioxidant activities of *C. dimorpha* extracts, a good correlation between the three methods was observed, which suggests that *C. dimorpha* secondary metabolites are capable to scavenge free radicals and reduce oxidants, where it was found that *CD*-EtOAc extract displayed significant radical scavenging and oxidant reducing activity compared to *CD*-BuOH extract. Although, the studied extracts had good DPPH radical scavenging activity, the recorded values when using this test were significantly lower than those when using phenanthroline and phosphomolybdenum assays (Figure 2). This could be explained by the fact that the mechanisms of action of the three methods are different, and the limited steric accessibility to the active site of the DPPH could decrease the anti radicalar efficacy (Xie & Schaich, 2014).

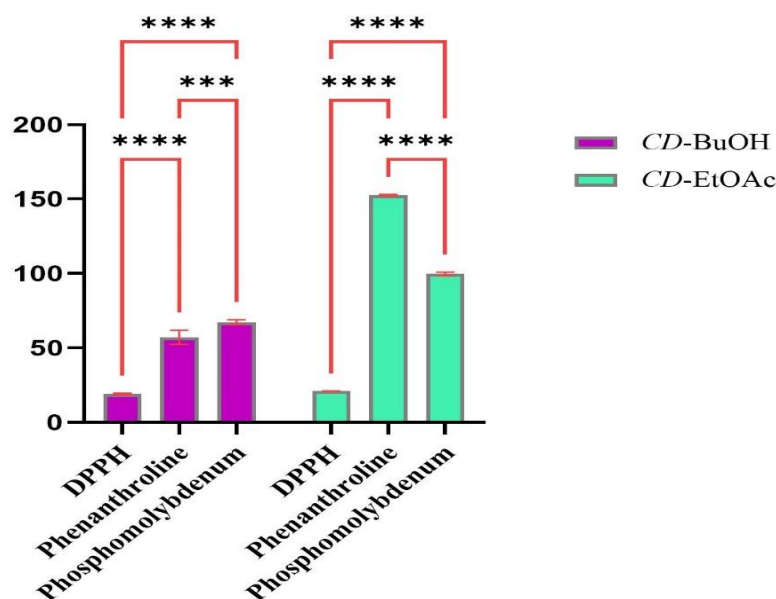


Figure 2. Comparison between antioxidant mechanisms. (\*\*\*\*) and (\*\*\*): Significant difference at  $p < 0.0001$  and  $0.001$

In the current study, *C. dimorpha* extracts showed significant anti-radicalar and reducing effects. Similarly, Bibi and coauthors (Bibi et al., 2024), noted that extracts of *Centaurea iberica* species exhibited significant DPPH scavenging activity and reducing power. Also, Yirtici and coauthors (Yirtici et al., 2022) examined the DPPH and phosphomolybdenum activities of *Centaurea sivasica* species and noted that this taxon displayed high activity. In addition, several authors confirmed the radical scavenging and reducing potential of plant extracts belonging to other species (Ayad et al., 2022; Boulacel et al., 2019; Rajurkar & Hande, 2011; Salachna et al., 2021). Consequently, *C. dimorpha* extracts can be used as natural antioxidant agents in several anti-aging formulations.



### 3.3 Photoprotective activity

#### 3.3.1 UV absorbing profile

Extracts obtained from plants contain a variety of phytoconstituents, which have UV absorption properties, particularly phenolics and flavonoids. This makes them a promising ingredient for designing new eco-friendly sunscreen formulations.

The results in (Figure 3) show that *C. dimorpha* extracts have the same UV absorption profile. In fact, they absorb both UVB and UVA radiation, but CD-EtOAc recorded high absorption in both UVB and UVA range. Based on this, it appears that these extracts could be promising agents with photoprotective capacity.

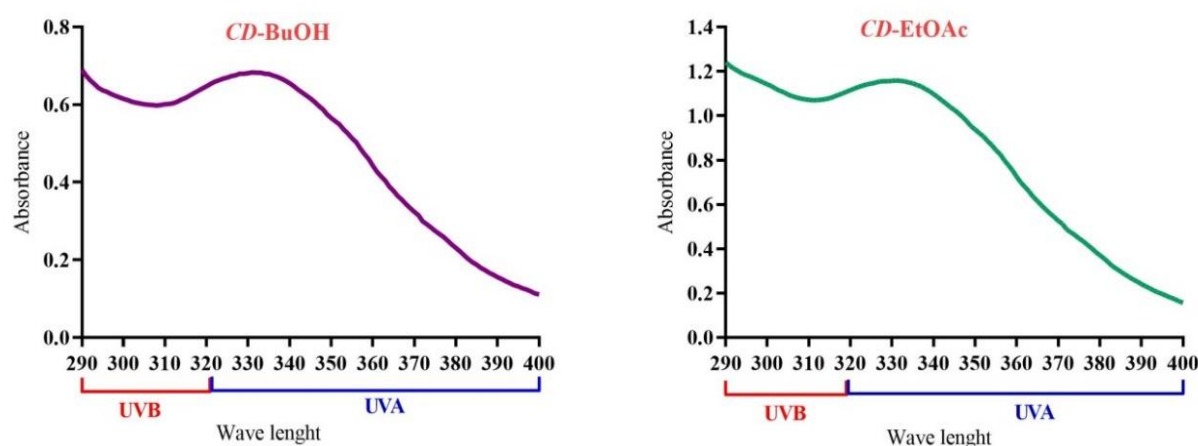


Figure 3. UV absorption profile of *C. dimorpha* extracts

#### 3.3.2 In vitro SPF estimation

SPF values (Table 2) showed that the two extracts have UVB photoprotection efficacy since they have met the regulation ruled by the European Commission categories ( $\text{SPF}_{\text{in vitro}} \geq 6.0$ ) (Seregheti et al., 2020).

Table 2.  $\text{SPF}_{\text{in vitro}}$  of *C. dimorpha* extracts and Benzophenone 3

Samples	$\text{SPF}_{\text{in vitro}}$
CD-EtOAc	$12.30 \pm 0.001^{***}$
CD-BuOH	$6.82 \pm 0.001^{***}$
Benzophenone 3	$36.43 \pm 0.001^{***}$

(\*\*\*): Significant difference at  $p < 0.001$ .

It can be also observed that CD-EtOAc extract ( $\text{SPF}_{\text{in vitro}} = 12.30 \pm 0.001$ ) was more effective UVB screens than CD-BuOH extract ( $\text{SPF}_{\text{in vitro}} = 6.82 \pm 0.001$ ). However, these values were significantly lower than that of Benzophenone 3 ( $\text{SPF}_{\text{in vitro}} = 36.43 \pm 0.001$ ), indicating that CD-EtOAc and CD-BuOH absorb UVB less than the standard. In a study performed by Bensaad and co-authors (Bensaad et al., 2021) the SPF values of the ethyl-acetate and Butanolic extract of *C. tougourensis* ( $\text{SPF} = 8.382$  and  $56.035$ , respectively at the concentration of  $4 \text{ mg mL}^{-1}$ ) were higher than those recorder in the current study. But the results of the *Centaurea dimorpha* are promising since the extract's concentration was very lower ( $0.1 \text{ mg mL}^{-1}$ ).

Furthermore, when comparing the antioxidant activity results of *C. dimorpha* extracts with their respective SPF values, a correlation was observed between the antioxidant effect and SPF values of *CD*-EtOAc and *CD*-BuOH extracts. This trend was also observed in the correlation between TPC, TFC and SPF *in vitro* values. Similarly, several authors revealed a good correlation between SPF *in vitro*, phenolic, flavonoid contents and antioxidant efficacy of various plant species (Lefahal et al., 2021; Mouffouk et al., 2020; Lassoued et al., 2021; Nunes et al., 2018).

Considering the universal cosmetic regulations, and our outcomes, it seems that the *C. dimorpha* extracts especially *CD*-EtOAc can be incorporated in sunscreen formulation as a promising UVB photoprotective ingredient.

### 3.3.3 UV broad-spectrum protection efficacy evaluation

The measured indices, UVA/UVB ratio and CW, for *CD*-EtOAc and *CD*-BuOH extracts are indicated in (Table 3).

Table 3. UVA/UVB ratio and CW of *C. dimorpha* extracts

Samples	UVA/UVB ratio	CW (nm)
<i>CD</i> -EtOAc	$0.63 \pm 0.001^{***}$	$371 \pm 0.00$
<i>CD</i> -BuOH	$0.69 \pm 0.001^{***}$	$372 \pm 0.00$

CW: Critical wavelength

The UVA/UVB ratio is used to evaluate the protection against UVA radiation, and according to their values the levels of the protection are classified (Kostyuk et al., 2018). The results showed that both *CD*-EtOAc and *CD*-BuOH extracts displayed a UVA/UVB ratio of  $0.63 \pm 0.001$  and  $0.69 \pm 0.001$ , respectively. These values indicate that the studied extracts are effective UVA protectants.

According the US-FDA, all products with CW  $\geq 370$ nm are considered as broad-spectrum protectants (Wang et al., 2017). Our study's results (Table 3) indicate that both *CD*-EtOAc and *CD*-BuOH have wavelengths values greater than 370 nm, which correlates with FDA's requirement for broad-spectrum protection. In this sense, *C. dimorpha* extracts have the potential for the development of photoprotective cosmetic formulations.

## 4. Conclusion

In an effort to identify new potential applications of *Centaurea dimorpha* Viv plant as a new cosmetic active ingredient, the current study concluded by revealing, for the first time, the polyphenolic content, antioxidant and photoprotective capabilities of this plant. According to the study's results, *CD*-EtOAc extract had significant levels of TPC, TFC and antioxidant activity. In addition, *CD*-EtOAc extract showed a promising photoprotective effect as a broad-spectrum UV filter. These results suggest that this extract is a promising antioxidant and broad-spectrum UV filter active ingredient for the development of sunscreen cosmetic preparations. However, further *in vivo* and *in vitro* studies should be conducted on *CD*-EtOAc extract to test its toxicity such as skin and eyes irritation to ensure that it is safe for consumers when incorporated into cosmetic formulation.

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## Author's declaration

The authors declare that there are no conflicts of interest related to this article. AS, LM, ME and LS carried out laboratory work and analysed data. SA and KM advised about the laboratory technique and conducted manuscript proofreading before sub-mission. All authors read and approved the final version of the manuscript.

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