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# *Helosciadium crassipes* Koch (Apiaceae) extracts as natural sunscreen and preservative additives

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#### Abstract

Recently, ingredients derived from natural sources have gained increasing interest in the cosmetic field due to their low toxicity. Therefore, this study was designed to explore the cosmetic potential of *Helosciadium crassipes* Koch (Apiaceae) species growing in Algeria as a promising natural preservative and broad-spectrum UV protection additive. In our study, the *in-vitro* antioxidant effect was assessed via DPPH radical scavenging and total antioxidant capacity by phosphomolybdenum method (TAC), while the protection against UVB radiation was evaluated according to the sun protection factor (SPF) by using UV spectroscopic technic at wavelengths ranging from 290 to 320 nm and Mansur's equation, for the photoprotective effect against long-wavelength UVA, UVA/UVB and critical wavelength ( $\lambda_c$ ) parameters were evaluated. The outcomes showed that among the tested extracts, the methanolic extract (MeOH) contains high levels of phenolics and flavonoids, and possesses a significant antioxidant effect, particularly in DPPH radical scavenging assay. Similarly, this last one exhibited high photoprotective activity in UVB and UVA ranges. The gathered results reveal the possibility of using this extract as a good natural additive to be incorporated into cosmetic formulations as a broad-spectrum UV protection candidate and as a preservative agent.

Keywords: Apiaceae; critical wavelength; HPLC; photoprotective activity; SPF; UVA/UVB

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#### Introduction

Currently, the novel tendency in cosmetic products design results in the use of natural ingredients, therefore medicinal and aromatic plants as well as marine algae have attracted a great attention to be used as a promising source of antioxidants and UV protectants in cosmetic preparations. The excessive exposure to sun's ultraviolet radiations has a damaging effects on human skin, they are able to induce erythema, sunburns and premature aging of skin (Stevanato et al., 2014). Therefore, it is necessary to protect the human skin from UV radiation, and one of the most prevention methods is based on the use of preparations containing UV absorbing agents (Leccia et al., 2019). On the other hand the excessive usage of UV filter agents as photoprotective ingredients has been extensively questioned in term of their health risks especially toxicity (Marto et al., 2016). For example, Benzophenone 3 an UV filter widely used as active ingredient in sunscreen products (Dodson et al., 2020) has significant toxicity toward humans and animals. Recent in vivo studies reported that the exposure to high levels of Benzophenone 3 could cause unusual change in the birth weight and gestational age of girls and boys, on the other hand the cytotoxicity of this UV filter was also observed for female mice, where the administration of highest concentrations resulted in an increased estrous cycle (Utsunomiya et al., 2019). Additionally, it was currently reported that synthetic UV filters have an impact on aquatic organisms. Actually, Benzophenone oxide and Benzophenone 3 were found to affect the coral growth (Yeo *et al.*, 2022).

The preservation of the cosmetic products as well as the protection of the consumer's health has gained increasing interests, for that several synthetic antioxidant preservatives are currently used in cosmetic and dermatologic formulations. Several studies have reported that antioxidants are incorporated into cosmetics owing to their ability to scavenge free radical, which could protect the human skin against the oxidative damage caused by ultraviolet radiation and by free radicals (Pisoschi *et al.*, 2016). Additionally, they are also used to avoid oxidative damage of active ingredients and oily constituents present in the cosmetic formulations (Cherubim *et al.*, 2020)

Actually, BHA and BHT are powerful synthetic antioxidant preservatives extensively used in different cosmetic preparations such as creams and lipsticks (Alvarez-Rivera *et al.*, 2018). Despite their significant potential in protecting against oxidant effect, these well-known synthetic preservatives are considered to be harmful for human's health, they are found to be carcinogenic and endocrine disruptors (Fayeulle *et al.*, 2021). Thus, it is necessary to find new active ingredients from bio-sources with less harmful effects to consumers to replace and be alternatives to synthetic preservatives (Alvarez-Rivera *et al.*, 2018). In this context plant extracts have attracted the attention of cosmetic formulators to test their applications in cosmetic preparations owing to their preservation effect especially antioxidant potential (Thibane *et al.*, 2019).

Apiaceae family consists of aromatic and medicinal plants that are commonly used in several economical fields such as cosmetic and cosmeceutical industries, they are considered to be a rich source of natural antioxidants (Thiviya *et al.*, 2021). Furthermore, extracts derived from Apiaceae species are used as natural sunscreening agents in cosmetic formulations. Previous study reported that extracts from Carrot and coriander are added in sunscreens because of their phenolic compounds (Sarkar *et al.*, 2013).

Algerian Apiaceae plants have attracted great interest as substantial source of bio additives which could be used for designing new cosmetic formulations, particularly those with antioxidant and anti-aging effect. Taking into account that there is no reported data published yet in term of the cosmeceutical potential of the species *Helosciadium crassipes* Koch (Apiaceae) growing in Algeria, this work was designed to evaluate the phytochemical characterization of the dichloromethane, ethyl acetate and methanolic extracts, in addition to their antioxidant and photoprotective activity in order to check the possible use of this species as promising additives in cosmetic formulations.

# Materials and Methods

#### Plant material

*H. crassipes* aerial parts were collected in March 2018 from Elkala in the east of Algeria, and identified by Prof. H. Laouer (Biology and Plant Ecology Department, University of Setif, Algeria). A voucher specimen was deposited in the Herbarium of our laboratory.

#### Extracts preparation

Powdered aerial parts of *H. crassipes* were successively extracted with different solvents in the increasing polarity. One hundred gram of powdered aerial parts was macerated in dichloromethane (DCM) in ratio (1/10) at room temperature for 72 h. The macerate was filtered and concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator producing dichloromethane extract. While the resulting residue was air dried and then extracted with ethyl acetate followed by methanol similar to the procedure adopted for dichloromethane extract preparation. Finally, the obtained extracts (DCM, EtOAc and MeOH) were weighted and kept at 4 °C till use.

#### Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), ferric chloride, ammonium molybdate tetrahydrate, sulfuric acid, Folin–Ciocalteu reagent, aluminum chloride, sodium carbonate, sodium hydroxide, gallic acid, quercetin, ascorbic acid was obtained from Sigma Aldrich (Steinem, Germany). Other chemicals used including the solvents were of analytical grade.

#### Phytochemical characterization

#### Total phenolic content (TPC) determination

The TPC was determined using Folin-Ciocalteu reagent (Boulacel *et al.*, 2019). Firstly, 300  $\mu$ L of diluted solution of H. crassipes extracts was added to 1500  $\mu$ L of Folin–Ciocalteu reagent (diluted 10-fold). The obtained mixture was neutralized by 1200  $\mu$ L of 7.5% Na<sub>2</sub>CO<sub>3</sub> after 4 min, and incubated at room temperature in the dark for 2 h, the absorbance was measured at 765 nm. All measurements were done in triplicate and the results expressed as gallic acid equivalents (mg GAE/g extract).

#### Total flavonoid content (TFC) determination

The TFC was determined as indicated in literature (Ayad *et al.*, 2017). Firstly, 1 mL of 2% AlCl<sub>3</sub> solution was mixed with 1 mL of diluted solution of *H. crassipes* extracts. Then the prepared mixture was incubated for 10 min at room temperature in dark, and the absorbance was recorded at 415 nm. All measurements were done in triplicate and the results expressed as quercetin equivalents (mg QE /g extract).

#### High-pressure liquid chromatography- diode-array detection (HPLC-DAD) analysis

The phytoconstituents composition analysis of *H. crassipes* extracts was performed according to the methodology described by Fedoul *et al.* (2022). The detection of these phytoconstituents was performed using an HP-Agilent 1290 Infinity HPLC equipped with a  $C_{18}$  column and diode array detector DAD. The elution phase consists of 3% acetic acid in water (A) and 100% methanol (B), with the gradient as follow: 93% A-7% B (0.1 min), 72% A-28% B (20 min), 75% A-25% B (8 min), 70% A-30% B (7 min); and the same gradient for 15 min was 67% A-33% B (10 min), 58% A-42% B (2 min), 50% A-50% B (8 min), 30% A-70% B (3 min), 20% A-80% B (2 min), and 100% B for 5 min until it reached the end of the run. The flow rate was 0.8 mL/min, the injection volumes were 1  $\mu$ L, and the extract concentrations were 20 mg/mL. The tested samples were prepared in methanol, and 20  $\mu$ L was the injecting volume. The detection wavelengths were set at 278 nm.

While, gallic, chlorogenic, caffeic, 3-hydroxybenzoic, syringic, coumaric, trans-ferulic, sinapic, benzoic, rosmarinic, cinnamic acids, catechin, epicatechin, catechin hydrate, hesperidin and quercetin were used as standards for comparison. The amount of individual phytoconstituent is expressed as mg per gram (mg/g) of extract.

# In vitro antioxidant activity analysis DPPH radical scavenging assay

The anti-radical activity was evaluated by DPPH assay (Almeida *et al.*, 2011). Concisely 400  $\mu$ L of diluted solution of *H. crassipes* extracts was added to 1600  $\mu$ L of 0.004% DPPH radical solution. The mixture was incubated for 30 min in the dark, and then the absorbance was measured at 517 nm.

#### Phosphomolybdenum assay

The total antioxidant capacity (TAC) was evaluated by phosphomolybdenum assay (Cherfia *et al.*, 2020). Three hundred microliter of diluted solution of *H. crassipes* extracts was added to 3 mL of reagent solution consisting of (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate), and incubated in a water bath at 95 °C for 90 min, after that the absorbance was measured at 695 nm.

For anti-radical effect and total antioxidant capacity, the results are expressed as ascorbic acid equivalents (mg AAE/g) and the BHT was used as positive control, and the assays were done in triplicate.

# In vitro photoprotective effect analysis

# UVA and UVB filtering potential

The absorption spectrum of *H. crassipes* extracts and Benzophenone 3 (positive control) was recorded following the procedure from literature (Ayad *et al.*, 2022). Firstly, dilution solutions of 0.02% were prepared by dissolving 20 mg of *H. crassipes* extracts and the positive control in 100 mL of methanol. Then spectrophotometric scanning in the range 290-400 nm was performed by using standard quartz cuvette and methanol as blank.

# In vitro sun protection factor (SPF) determination

For SPF parameter determination, *H. crassipes* extracts and Benzophenone 3 were diluted in absolute methanol to obtain concentration of 0,2 mg/mL, and then the absorbance was recorded every 5 nm at wavelengths ranging from 290 to 320 nm with UV/VIS spectrophotometer.

The measurements were performed using standard quartz cuvette and methanol as blank.

The SPF parameter was determined using Mansur's equation (Vostálová et al., 2019).

SPFspectrophotometric = 
$$CF \sum_{290}^{320} EE(\lambda)I(\lambda)abs(\lambda)$$

EE ( $\lambda$ ): erythemogenic effect of radiation, I ( $\lambda$ ): solar intensity, and CF (= 10): correction factor are constant (Sayre *et al.*, 1979). Abs ( $\lambda$ ): absorbance.

<u>Critical wavelength ( $\lambda_c$ )</u>

This parameter was calculated using the equation developed by Diffey (Diffey, 2007).

$$\int_{290}^{\lambda c} A(\lambda) d(\lambda) = 0, 9 \int_{290}^{400} A(\lambda) d(\lambda)$$

A ( $\lambda$ ): absorbance at wavelength  $\lambda$ . The broad spectrum photoprotection is considered when  $\lambda_c \ge 370$  nm (Caballero-Gallardo *et al.*, 2022). No protection against UV radiation when  $\lambda_c < 325$  nm (Kurzawa *et al.*, 2022).

UVA/UVB ratio

This ratio is expressed as the mean UVA absorbance to the mean UVB absorbance, and calculated as follow:

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

A ( $\lambda$ ): absorbance at wavelength, d $\lambda$  is the wavelength increment (1 nm)(Ferrero *et al.*, 2010).

The UVA/UVB ratio of our samples was checked at the dilution of 0.02% (Kostyuk et al., 2018).

According to this ratio values the star rating system indicates that the UVA/UVB ratio in the range (0.0-0.2): too low for UVA protection (-), (0.2-0.4): moderate UVA protection (\*), (0.4-0.6): good UVA protection (\*\*\*), (0.6-0.8): superior UVA protection (\*\*\*), and  $\geq 0.8$ : maximum UVA protection (\*\*\*\*).

#### Statistical analysis

Statistical analysis was applied using one way analysis of variance (ANOVA) to assess significant differences between samples, values of p <0.05 were considered significant. All statistical analyzes were performed using the Graphpad prism application.

#### **Results and Discussion**

#### Phytochemical characterization

In this study three solvents of different polarities (Dichloromethane <Ethyl acetate <Methanol) were used to extract phenolics and flavonoids present in aerial parts of *H. crassipes* species, and the amounts of this metabolites were spectrophotometry quantified. The results (Table 1) revealed that the MeOH extract was found to contain a significant amount of phenolics,  $(22.85 \pm 0.35 \text{ mg GAE /g extract})$  compared to EtOAc and DCM extracts  $(14.57 \pm 0.59 \text{ mg GAE /g extract} and 5.37 \pm 0.94 \text{ mg GAE /g extract}, respectively)$ . Likewise, for TFC the MeOH extract had the highest amount of flavonoids  $(20.50 \pm 0.08 \text{ mg QE /g extract})$ . It can also be observed that the most polar solvent methanol was found to be more effective for extracting *H. crassipes* phenolics and flavonoids. Thus our findings are in line with those of several authors reporting the effectiveness of methanol for extracting and obtaining a high amount of phenolics and flavonoids (Dall'Acqua *et al.*, 2022).

Samples	TPC (mg GAE /g extract)	TFC (mg QE /g extract)		
DCM	5.37 ±0.94"	6.97±0.42 <sup>ns</sup>		
EtOAc	14.57 ±0.59"	8.20±0.83 <sup>ns</sup>		
MeOH	22.85 ±0.35***	20.50±0.08***		

Table 1. Total phenolic and flavonoid contents of the H. crassipes extracts

Values are means  $\pm$  SD (n=3). TPC: y= 0,01435x+0,2567 (R<sup>2</sup>=0,9929). TFC: y= 0,02554x+0,0078 (R<sup>2</sup>=0,9965). (""): significant difference at p<0.0001 between samples. (ns): no significant difference at p<0.05 between samples.

#### High-pressure liquid chromatography- diode-array detection (HPLC-DAD) analysis

Identification and quantification of phytoconstituents detected in *H. crassipes* extracts was performed by HPLC-DAD, and the results are shown in (Table 2).

Peak	Identification	DT ()		Quantity mg/g extract		
	Identification	RT (min.)	Correlation (r <sup>2</sup> )	EtOAc	MeOH	
1	3-Hydroxy benzoic acid	22.545	0.99928	19.898	6.175	
2	Catechin hydrate	11.499	0.99906	5.196	nd	
3	Chlorogenic acid	16.239	0.99970	nd	17.51	
4	Caffeic acid	21.476	0.99892	nd	5.613	
5	Epicatechin	20.169	0.99879	252.153	28.527	
6	Gallic acid	5.912	0.99966	nd	0.502	
7	P-Coumaric acid	33.597	0.99982	1.454	1.062	
8	Rosmarinic acid	70.655	0.99907	nd	7.919	
9	Sinnapic acid	37.264	0.99925	2.464	Nd	
11	Syringic acid	22.628	0.99839	6.622	2.341	
12	Trans-Cinnamic acid	75.207	0.99998	0.454	3.857	
13	Trans-Ferrulic acid	37.202	0.99993	1.679	nd	

Table 2. Individual phytoconstituents detected in H. crassipes extracts

nd: not detected

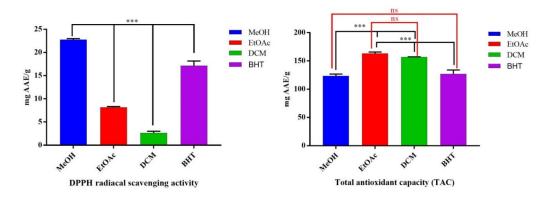
By assessing the results of the individual phytoconstituents detected in *H. crassipes* extract, it is showed that MeOH extracts contains 8 phenolic acids including: (3-Hydroxy benzoic, chlorogenic, caffeic, gallic, p-coumaric, rosmarinic, syringic and t-Cinnamic acid). Obviously, higher amount of chlorogenic acid (17.51 mg/g), rosmarinic acid (7.919 mg/g), 3-hydroxy benzoic acid (6.175 mg/g) and caffeic acid (5.613 mg/g) was found in this extract. Concerning the EtOAc extract 6 phenolic acids were detected, three among them are abundant: 3-hydroxy benzoic acid (19.898 mg/g), syringic acid (6.662 mg/g) and sinnapic acid (2.464 mg/g). In the light of these results, it can also be observed that the MeOH extract was much richer than EtOAc extract in term of phenolic acids amount. Looking at the overall results of the two *H. crassipes* extracts, a high amount of epicatechin (252.153 mg/g) was found in EtOAc extract.

It is worth noting that phenols and flavonoids present in plant extracts were found to act as free radical scavengers and may contribute straight forwardly to their antioxidant effect, and the presence of these phytoconstituents can promote the capability to absorb UVR radiations, which could justify the possibility to use the plant extracts as photoprotectant ingredients (Fardiyah *et al.*, 2020).

Consequently, the presence of these phytoconstituents in *H. crassipes* extracts could justify the antioxidant and photoprotective effects of this species.

# Analysis of antioxidant activity

The antioxidant proprieties of *H. crassipes* were studied via two antioxidant assays (DPPH and Phosphomolybdenum assay), and the (Figure 1) summarizes the results.



**Figure 1.** Antioxidant activities of *H. crassipes* extracts and BHT (Positive control) Values are means  $\pm$  SD (n = 3). DPPH: (mg AAE/g), y= -0.03461x + 0.8852 (R<sup>2</sup>=0.9957). TAC: Total antioxidant capacity (mg AAE/g), y= 0.00632x-0.00022 (R<sup>2</sup>=0.9902). <sup>(\*\*)</sup>: significant difference at p<0.001 between samples. <sup>(ns)</sup>: no significant difference at p<0.05 between samples.

Based on the obtained data, the most pronounced DPPH radical scavenger was the MeOH extract  $(22.76\pm0.23 \text{ mg AAE/g})$  followed by EtOAc extract  $(8.17\pm0.18 \text{ mg AAE/g})$  while the weakest DPPH radical scavenger was DCM extract  $(2.66\pm0.35 \text{ mg AAE/g})$ , the results also show that the MeOH extract was found to be more potent than BHT (antioxidant standard)  $(17.151\pm1.009 \text{ mg AAE/g})$ . It is worth noting that there is a correlation between anti-radical potential of plant extracts and their phenolic and flavonoids composition (Hossain *et al.*, 2012), therefore the significant DPPH radical scavenging ability of *H. crassipes* MeOH extract could be attributed to the highest phenols and flavonoids amounts. These results are consistent with previous reports on other Apiaceae species (Lefahal *et al.*, 2018; Zengin *et al.*, 2019).

When examining the results of the total antioxidant capacity (TAC), it was observed that both EtOAc and DCM extracts exhibited substantial efficacy, with  $163.29\pm2.58 \text{ mg} \text{AAE/g}$  and  $157.02\pm0.45 \text{ mg} \text{AAE/g}$ , respectively. Additionally, the results show that the EtOAc and DCM extracts were found to be more potent than the positive control (BHT) in term of total antioxidant capacity, while the MeOH extract ( $123.37\pm3.59 \text{ mg} \text{AAE/g}$ ) had similar effect to that of BHT. It is clearly shown that there is no correlation between DPPH and phosphomolybdenum assay (TAC). This result is in accordance with that previously reported by Dall'Acqua *et al.*, 2022.

In the light of these collected data, it was noticed that *H. crassipes* extracts possess significant antioxidant capacity; this effectiveness could be attributed to phenols and flavonoids present in these extracts. This accords well with previous studies that proved close relationship between the total antioxidant capacity of plant extracts and the phenolic and flavonoid composition (Bourgou *et al.*, 2008; Ayad *et al.*, 2022).

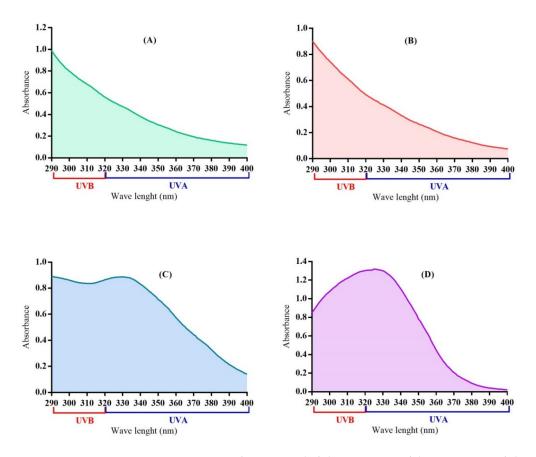
In recent years the novel trend in cosmetic field, is the use of antioxidant molecules derived from natural origin, as efficient alternatives to synthetic additives such as preservatives (Aguilar-Toalá *et al.*, 2019). Taking into account the recorded outcomes of our study concerning the antioxidant potential of *H. crassipes* extracts, it seems that the aerial part extracts of this species might be a promising natural preservative to be incorporated into several cosmetic preparations.

#### Photoprotective activity

#### UVA and UVB absorbing effect assessment

UV spectrums were recorded in order to evaluate the UV absorbing effect of *H. crassipes* extracts. As shown in Figure 2, it is clearly observed that all tested samples have the ability to absorb UVB and UV-A radiations. It can also be observed that the MeOH extract was more effective in UV-A and UV-B absorbing

potential compared to EtOAc and DCM extracts. Therefore, it seems that this extract could be a promising photoprotectant ingredient.



**Figure 2**. The absorbance within the range of (290–400 nm). (A): DCM extract, (B): EtOAc extract, (C): MeOH extract, (D): Benzophenone 3

# Determination of sun protection factor (SPF), protection from UVA and critical wavelength

The SPF values of *H. crassipes* extracts were determined using Mansur's equation. It is clearly shown that the SPF parameter is directly proportional to the concentrations of the tested samples (Figure 3), and the increasing of sample's concentration maximizes SPF value.

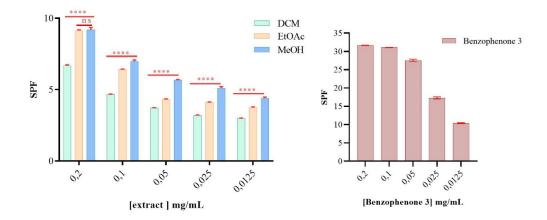


Figure 3. Sun protection factor of H. crassipes extracts and Benzophenone 3 Values are means  $\pm$  SD (n=3). (\*\*\*\*) indicates a significant difference at p<0,0001 between samples.

Actually, the SPF values of DCM, EtOAc and MeOH extracts achieved  $6.67\pm0.07$ ;  $9.15\pm0.04$  and  $9.19\pm0.16$  respectively at the highest concentrations (200 µg/mL), while in the lowest concentrations (12.5 µg/mL), SPF values were  $3.00\pm0.01$ ;  $3.77\pm0.02$  and  $4.40\pm0.08$  respectively. The data also showed that MeOH and EtOAc extracts were more effective UVB screens compared to the DCM extract, but at the same time they absorb UVB radiations less effectively than Benzophenone 3 (SPF=  $31.67\pm0.06$ ;  $10.40\pm0.15$  respectively).

The effectiveness of *H. crassipes* extracts in term of photoprotective potential could be explained by their richness in phenolic and flavonoid compounds. Taking into account this observation, our collected results are in line of those of several authors associating the UV absorbing potential of plant extracts with their phytoconstituents particularly phenols and flavonoids (Amrani *et al.*, 2019; Mouffouk *et al.*, 2020; Yakoubi *et al.*, 2021).

The cosmetic universal recommendations consider a photoprotection product as a true sunscreen, all compound whose the minimum SPF value is 6 (Seregheti *et al.*, 2020), taking into account these recommendations and the fact that the SPF values of our study could reach levels greater or equally 6 at lower concentration (0.02%, w/v), the *H. crassipes* extracts could be a promising sun screening ingredients.

In terms of the protection from UVA radiation, the collected data (Table 3) demonstrated that all samples were found to be effective toward UVA radiation with different levels, the DCM, EtOAc extracts had low protection against UVA radiation (one star), and Benzophenone 3 had good protection (two stars), while the MeOH extract had superior protection (three stars).

Samples		CM	EtOAc		MeOH		Benzophenone 3	
UVA/UVB ratio	0.38	*	0.34	*	0.65	***	0.49	**
Critical wavelength (λ <sub>c</sub> )	330.21		326		371		355	

**Table 3.** UVA/UVB ratio and Critical wavelength ( $\lambda_c$ ) values calculated from *H. crassipes* extracts and Benzophenone 3 (positive control)

Interestingly, the MeOH extract absorbs predominantly UVA compared to Benzophenone 3 (positive control), this may be explained by the presence of high amount of phenols like; chlorogenic, caffeic, gallic, p-coumaric, rosmarinic, syringic and t-Cinnamic acid, this in line with previous reports that prove the effectiveness of phenolic acids in screening UVA light compared to benzophenone-3 (Kostyuk *et al.*, 2018).

Concerning the critical wavelength ( $\lambda_c$ ), the results indicated that all *H. crassipes* extracts and Benzophenone 3 presented protection against UV radiation since critical wavelength's values were higher than 325 nm, but MeOH extract was more effective in UVA absorbing potential (critical wavelength,  $\lambda_c \ge 370$  nm). Consequently, the data that we obtained suggest that the MeOH extract could be a promising broad spectrum UV protection candidate.

#### Conclusions

In this study, phytochemical characterization, antioxidant and photoprotective potential of *H. crassipes* extracts were checked for the first time in an attempt to unravel new possible uses of this species in cosmetic field. Overall, the obtained results showed that the MeOH extract show good protection against UV radiation, thus it could be a promising candidate for incorporating into cosmetic preparations, particularly in sunscreen formulations for improving the protection against UV radiation. The findings of photoprotective activity (SPF, UVA/UVB and critical wavelength) for extracts at the concentration only of 0,02% w/v are very promising and could have the potential to be developed as sunscreening agent. For all extracts the antioxidant activity was evaluated through DPPH and phosphomolybdenum methods. These entire candidates showed the highest antioxidant capacity, even greater than that of the synthetic antioxidant BHT. Hence *H. crassipes* extracts are promising ingredients to be incorporated as natural antioxidant preservatives into cosmetics. However, further *in vivo* and *in vitro* investigations should be achieved to test their toxicity to be safe for consumers when incorporated into cosmetic formulations.

#### Authors' Contributions

Conceptualization: EHM, ML; Data curation: RA; Formal analysis: EHM, RA; Investigation: SA; Methodology: EHM, RA, ML, YSC; Resources: SA; Supervision: SA, GN; Validation: RA, ML; Writing - original draft: EHM; Writing - review and editing: SA, EHM. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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# **Conflict of Interests**

The authors declare that there are no conflicts of interest related to this article.

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