

RESEARCH ARTICLE

Impact of drying methods on antioxidant capacity and anti-photoaging of *Cosmos caudatus* extract for potential cosmeceutical applications

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Abstract - *Cosmos caudatus* possesses well-known antioxidant properties. Nonetheless, the impact of drying after extraction on the preservation of antioxidants and the subsequent anti-photoaging activity has yet to be investigated. This study investigated the effects of different drying techniques on the antioxidant content and anti-photoaging activity of *C. caudatus* aqueous extracts. The extract was obtained by Soxhlet extraction (1:25 w/v, 8 h) and was dried using freeze, spray or oven drying prior to analysis. The extract's anti-photoaging effect was assessed in ultraviolet B (UVB)-irradiated HSF1184 fibroblasts using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay and the Sircol collagen assay. Freeze-drying yielded the highest antioxidant capacity by the 2,2-diphenyl-1-picrylhydrazyl assay (0.92 ± 0.020 mg ascorbic acid equivalent/g) and ascorbic acid (AA) content determined by high-performance liquid chromatography (0.69 ± 0.06 mg/g) compared with spray and oven drying. Treatment with the freeze-dried extract significantly enhanced collagen synthesis in the UVB-irradiated fibroblasts ($p < 0.05$), reaching 92.86% and 143.65% relative to the non-UV control at 100 $\mu\text{g/mL}$ and 500 $\mu\text{g/mL}$, respectively. The effect was comparable to the treatment with AA at 100 $\mu\text{g/mL}$ (97.85%). These findings demonstrate that freeze-drying effectively preserves AA content, while the resulting *C. caudatus* aqueous extract retains anti-photoaging activity, highlighting the critical role of drying methods in preserving bioactivity for potential cosmeceutical applications.

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1. Introduction

Vitamins are widely used as antioxidants in cosmetic formulations, including vitamin A (retinol), vitamin B3 (nicotinamide), vitamin C (ascorbic acid), vitamin E (tocopherol), and coenzyme Q10 [1]. Ascorbic acid (AA), in particular, is essential in anti-ageing treatments since it promotes collagen synthesis, antioxidant protection, and reduces melanin synthesis [2]. Research indicates that vitamin C levels in skin cells decline in aged or photodamaged skin due to oxidative stress [3]. This occurs when cells' oxidative activity and antioxidant capacity are imbalanced, causing reactive oxygen species (ROS) accumulation. The excess ROS generation and a decline in antioxidant activity contribute to intrinsic ageing, while increased ROS production due to external factors like exposure to pollutants and ultraviolet (UV) leads to extrinsic ageing. Moreover, studies have shown that inducing oxidative stress in fibroblasts inhibits collagenase activity, resulting in collagen degradation [4–5]. This inhibition contributes to skin ageing, making it crucial to include AA in skin care for its anti-ageing properties. Current skincare development increasingly emphasises natural extracts due to their safety, efficacy, and cost-effectiveness [6]. Many anti-ageing compounds are plant-derived, as they can restore procollagen levels and counteract ultraviolet B (UVB)-induced collagen loss comparable to AA [7].

Cosmos caudatus is a medicinal plant recognised for its antioxidant, anticancer, antidiabetic, and antimicrobial properties [8]. Its notable antioxidant capacity is largely attributed to its high AA content of 1.09 ± 0.01 mg/g in the extract, determined by the titrimetric method [9]. Several studies have reported that *C. caudatus* extract exhibits enzyme-inhibitory properties, including suppression of tyrosinase, collagenase, and elastase activities, suggesting anti-ageing potential [8, 10–11]. However, the efficacy of *C. caudatus* aqueous extract against photoaging, particularly its ability to stimulate collagen synthesis under UVB-induced skin ageing, remains unexplored. A recent study demonstrated a positive anti-ageing effect of *C. caudatus* ethanolic extract in UVB-exposed fibroblasts. However, its antioxidant capacity is mainly attributed to flavonoids (quercetin and quercetin), which is still lower compared to AA [12].

Additionally, post-extraction processing (e.g., drying) directly affects the quality of medicinal plant extracts [13]. In the extraction process, high temperatures are necessary to disrupt the plant cell structures and free polyphenols from the plants [14]. However, drying liquid extracts containing solvents such as ethanol or water is a critical processing step because inappropriate drying conditions may degrade heat-sensitive compounds, particularly AA, reducing antioxidant capacity. Thermal drying methods (e.g., spray, air, vacuum, and oven) are more economical, but they may degrade AA compared with freeze-drying. Although spray-drying may improve powder solubility and sensory quality, its effect on antioxidant retention is still variable [15–16]. Hence, it is vital to choose the right drying technique to preserve the bioactive compounds of *C. caudatus* extract and ensure its effectiveness as an anti-ageing agent in skin formulations. The effects of freeze-, spray-, and oven-drying on the antioxidant activity and AA retention of aqueous *C. caudatus* extracts. The anti-photoaging efficacy of the dried extracts was subsequently determined using UVB-irradiated HSF1184 human skin fibroblasts.

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2. Materials and Methods

2.1 Materials

HSF1184 fibroblasts were provided by Universiti Malaysia Terengganu, Malaysia. Cell culture reagents such as Dulbecco's Modified Eagle's Medium, fetal bovine serum, and penicillin–streptomycin were purchased from Invitrogen (California, USA) and the Sircol™ collagen assay was procured from Biocolor Ltd., UK. Chemical reagents such as (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were supplied by Sigma-Aldrich (USA), whereas AA was sourced from HmbG Chemicals (Hamburg, Germany).

2.2 Sample Preparation

Fresh *C. caudatus* leaves were collected from Kuantan, Pahang, Malaysia, and washed with distilled water. The leaves were then oven-dried at 40 °C for 24 h and ground into a powder using a grinder before extraction.

2.3 Soxhlet Extraction

The process was performed at the boiling point of distilled water (100 °C) via a Soxhlet extractor using a 1:25 (w/v) solid-to-solvent ratio for 8 h in one extraction cycle. This extractor was chosen because its continuous solvent reflux allows consistent recovery of antioxidants for evaluation of drying methods [17]. Before drying, the extract was filtered through Whatman No. 1 filter paper [18].

2.4 Post-Extraction Drying Methods

The extracted filtrate was dried using three different drying methods: freeze-drying (Biotron model Cleanvac 12), spray-drying (Autonic Lab Plant SD06), and oven-drying (Daniel AOVE 558). For freeze-drying, the aqueous extract was frozen at -20 °C for 24 h and then lyophilised at -40 °C for 30 h [19]. In spray drying, the extract was dried for 1 h using an inlet and outlet temperature of 130 °C and 85 °C, respectively [20]. For oven drying, the extract was dehydrated at 50 °C for 48 h [21]. After drying, all samples were kept in sealed containers at room temperature until further analysis.

2.5 Analysis

2.5.1 DPPH assay

We evaluated antioxidant capacity using the DPPH assay as described in [22]. Absorbance was recorded at 517 nm using a UV-Vis spectrophotometer (Thermo Scientific AquaMate 7100). Antioxidant capacity was calculated from an AA standard curve (0–2.0 mg/mL) and expressed as mg AAE/g sample.

2.5.2 HPLC analysis

AA was quantified by high-performance liquid chromatography (HPLC) following the method described in [23], using an Agilent 1200 HPLC system equipped with a C18 column (150 × 3.9 mm i.d., 5 µm). Chromatographic separation was conducted using 0.05 M potassium dihydrogen phosphate and methanol (70:30, v/v) as the mobile phase at a flow rate of 1 mL/min. Detection was performed at 245 nm, and AA was quantified using a calibration curve (0–0.8 mg/mL).

2.6 Cell Culture and Treatment

HSF1184 cells were cultured and treated following the standard protocol [24]. Briefly, cells were exposed to UVB irradiation at 312 nm (25 mJ/cm²) and then treated with *C. caudatus* extract at 100 µg/mL and 500 µg/mL. AA (100 µg/mL) served as the positive control, while non-irradiated cells were used for normalisation (100%) [25].

2.6.1 MTT assay

Cytotoxic effects were assessed using the standard MTT assay procedure with three biological replicates. After treatment, 10 µL of MTT stock solution was added into each well of the 96-well plate. The plates were kept at 37 °C for 4 h to allow for formazan crystal formation. Then, 200 µL of DMSO was introduced to stabilise the crystals, resulting in a purple solution. Absorbance was then recorded at 570 nm, using 630 nm as the reference wavelength [26].

2.6.2 Sircol collagen assay

Type I collagen content was quantified using the Sircol™ collagen assay, with three biological replicates. Briefly, collagen in the cell lysates was bound to the Sircol dye reagent, and the resulting pellet was dissolved in alkaline reagent before measuring the absorbance at 555 nm [25].

2.7 Statistical Analysis

We used Origin 2022 (OriginLab, USA) for statistical analysis. Experimental results are reported as mean ± SD. One-way ANOVA followed by Tukey's multiple comparison test was applied to assess differences among treatments, and statistical significance was accepted at $p < 0.05$.

3. Results and Discussion

3.1 Effect of Drying Methods on *C. caudatus* Aqueous Extract

Drying of the aqueous extract resulted in the loss of bioactive compounds. Table 1 illustrates the antioxidant capacity and AA content in the *C. caudatus* aqueous extract, as determined by the DPPH assay and HPLC analysis, respectively. All

drying methods significantly reduced the antioxidant capacity compared with the fresh extract (2.87 ± 0.11 mg AAE/g). The freeze-dried extract retained the highest antioxidant capacity (0.92 ± 0.02 mg AAE/g), followed by the spray-dried extract (0.71 ± 0.10 mg AAE/g) and oven-dried extract (0.51 ± 0.07 mg AAE/g). However, antioxidant capacity did not differ significantly among the freeze-, spray-, and oven-dried extracts ($p > 0.05$). These findings are consistent with earlier studies reporting that, although drying reduces antioxidant capacity compared with fresh extracts, the extent of the reduction does not differ significantly among drying methods [27–28]. Nevertheless, the significant decrease in antioxidant capacity following all drying methods highlights the need to enhance antioxidant stability throughout processing. Using natural biopolymer-derived stabilisers, such as carrageenan, pectin, gelatin, and chitosan, may be a promising approach to reduce the loss of bioactive compounds during drying [29].

Additionally, AA content showed a similar decreasing trend after drying: freeze-, spray-, and oven-dried extracts were 0.69 ± 0.06 mg/g, 0.61 ± 0.02 mg/g, and 0.43 ± 0.03 mg/g, respectively. The findings were also aligned with the HPLC-quantified AA of lyophilised *C. caudatus* extract, which was reported to range from 0.085 to 1.537 mg/g [30–31]. The results showed that freeze-drying, which involves freezing the sample at low temperature followed by sublimation of moisture under low pressure, caused minimal vitamin C degradation. Spray-drying showed intermediate effects, suggesting partial degradation during the thermal process. Likewise, previous studies have found no statistically significant differences in AA concentration, although freeze-dried extracts are slightly higher than spray-dried extracts [32–34]. In comparison, oven drying led to the lowest antioxidant capacity, likely because AA is thermosensitive and more susceptible to oxidative degradation during prolonged exposure to elevated temperatures [35]. While AA significantly contributes to the antioxidant activity of *C. caudatus*, previous findings also indicate that phenolic compounds, flavonoids, glycosides, and other antioxidant phytochemicals are susceptible to degradation during drying, which lowers total antioxidant activity [36]. Furthermore, HPLC-determined AA content was highly correlated with DPPH antioxidant capacity ($r = 0.998$), as indicated by Pearson's correlation analysis (Figure 1), supporting the validity of the results. Although the freeze- and spray-dried extracts did not differ significantly concerning the antioxidant properties, the freeze-dried *C. caudatus* extract retained the highest AA content and was therefore selected for evaluation in UVB-irradiated fibroblasts.

Table 1. Influence of drying methods on the antioxidant capacity and ascorbic acid content in *C. caudatus* aqueous extract. The methods that do not have a common letter are markedly different

Drying process	Antioxidant capacity (mg AAE/g)	Ascorbic acid content (mg/g)
Before drying	2.87 ± 0.11	2.38 ± 0.07
Freeze	0.92 ± 0.02^a	0.69 ± 0.06^a
Spray	0.71 ± 0.10^{ab}	0.61 ± 0.02^{ab}
Oven	0.51 ± 0.07^b	0.43 ± 0.03^b

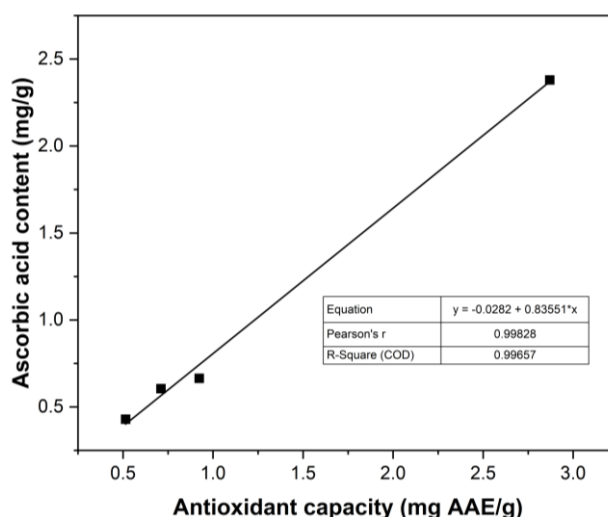


Figure 1. Correlation between ascorbic acid content (mg/g) and antioxidant capacity (mg AAE/g) of *C. caudatus* aqueous extract subjected to different drying methods

3.2 Cytotoxic Effect on UVB-Irradiated Fibroblasts

As illustrated in Figure 2, treatment of UVB-irradiated cells with 100 $\mu\text{g/mL}$ and 500 $\mu\text{g/mL}$ of lyophilised *C. caudatus* aqueous extract improved cell viability, restoring viability to 88.79% and 97.90%, respectively, comparable to that of the positive control (AA, 95.11% at 100 $\mu\text{g/mL}$). In contrast, UVB-exposed cells maintained in serum-free media without treatment showed reduced viability, reflecting the damaging effects of UVB-exposure. These results indicate that *C. caudatus* extract is non-cytotoxic at the tested concentrations and may support fibroblast recovery after UVB-induced oxidative stress. This finding aligns with earlier research demonstrating that plant extracts promote cell recovery after

UVB exposure, although the reported recovery percentages vary. For instance, treatment with a low concentration of *Labisia pumila* extract (0.5-10 µg/mL) enhanced cell viability recovery to 72-92% following UVB exposure [25]. In another study, 100 µg/mL *Gardenia jasminoides* extract restored approximately 75% cell viability in UVB-damaged keratinocytes [37]. More recently, treatment with *Zea mays* extract at 1000 µg/mL significantly increased cell recovery to 80% compared with UVB-exposed keratinocytes [38].

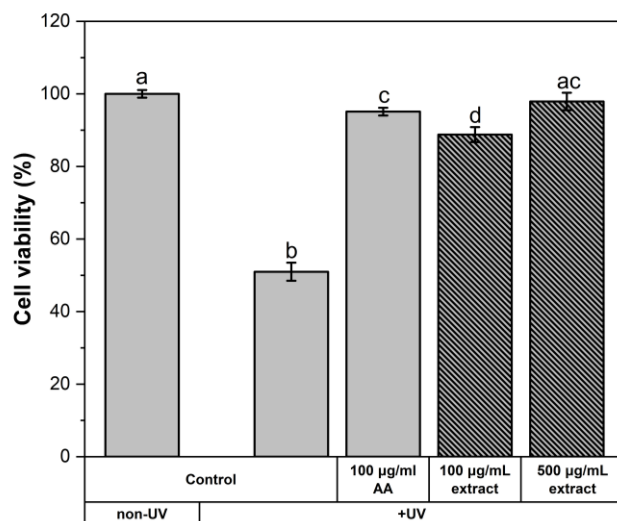


Figure 2. The effect of *C. caudatus* aqueous extract on cell viability in UVB-irradiated HSF1184 cells. Values are normalised to the non-UVB, untreated control (100%). Different superscript letters (a–d) indicate significant differences among treatments ($p < 0.05$), while shared letters indicate no significant difference

3.3 Collagen Synthesis Enhancement in UVB-Damaged Fibroblasts

The lyophilised aqueous extract of *C. caudatus* was tested using the Sircol collagen assay to assess whether it promotes the type I collagen synthesis in fibroblasts after UVB exposure. Figure 3 shows that 100 µg/mL and 500 µg/mL of extract enhanced collagen synthesis to 92.86% and 143.65%, respectively, relative to the non-UV control cells. However, without antioxidants, collagen synthesis in UV-treated cells decreased to 80.73% relative to the non-UV control. The observed enhancement of collagen synthesis by *C. caudatus* extract is consistent with previous studies demonstrating the collagen-promoting effects of antioxidant-rich extracts following UVB exposure. For instance, the aqueous extract of *Dracocephalum moldavica* (125 µg/mL) outperformed AA in stimulating procollagen in UVB-irradiated HaCaT keratinocytes [39]. Similarly, microalgal extracts at 500 µg/mL significantly restored collagen levels by 80% in the UVB-exposed Hs68 human fibroblasts, comparable to AA [40]. These findings show that the extracts promoted type I collagen synthesis in UVB-damaged fibroblasts. The increased collagen production observed at 500 µg/mL compared with the AA positive control may be due to the complex phytochemical composition of the *C. caudatus* extract. In addition to AA, *C. caudatus* contains phenolic compounds and flavonoids that may work together to improve fibroblast activity and extracellular matrix production. Interactions among various bioactive components may enhance collagen synthesis more effectively than AA alone [41]. Nonetheless, additional research is needed to identify the specific compounds and molecular mechanisms that drive this effect.

While the current study did not assess ROS production, earlier research has shown that UVB exposure elevates intracellular ROS levels, which then activate the matrix metalloproteinase expression (MMPs), including the main enzyme that facilitates collagen degradation (MMP-1) [4–5]. Furthermore, UVB triggers fibroblasts to produce MMP-3 and MMP-9, which further degrade collagen synthesis [42]. The results also indicate that the increase in collagen synthesis by the AA is due to its role in reducing ROS and thus inhibiting MMP-1 activity [43]. According to a recent study, a lower concentration of 25 µg/mL ethanolic extract of *C. caudatus*, containing 11.20% (w/w) quercetin and 14.79% (w/w) quercitrin, successfully inhibited collagenase activity and the protein expression of MMP-1 and MMP-3 in fibroblast cells [12]. However, the effect on collagen synthesis was not determined. In another study, collagenase activity was inhibited by 51.3% with treatment using 1000 µg/mL *C. caudatus* water extract, as assessed by an enzymatic assay, although its efficacy against UVB irradiation was not determined. Therefore, the aqueous extract of *C. caudatus* may reduce ROS production and inhibit MMP activity, potentially preventing UVB-induced collagen breakdown. To validate the mechanism by which this aqueous extract suppresses collagenase activity and protein expression, further research is necessary.

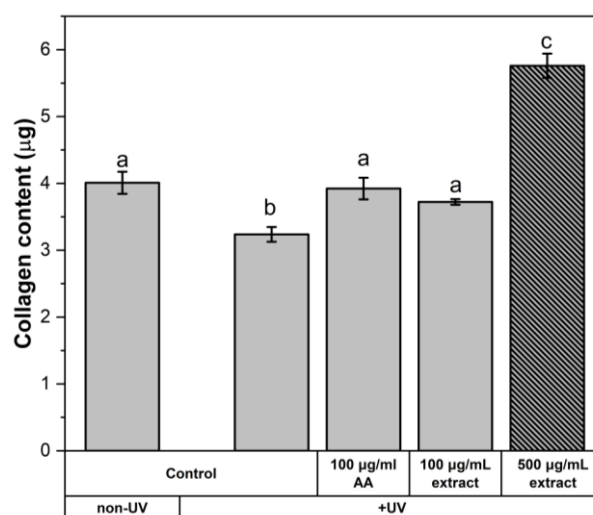


Figure 3. The effect of *C. caudatus* aqueous extract on collagen synthesis in HSF1184 cells. Different superscript letters (a–d) indicate significant differences among treatments ($p < 0.05$), while shared letters indicate no significant difference

4. Conclusions

In summary, the study suggests that an aqueous extract of *C. caudatus* exhibits antioxidant and anti-photoaging effects, as demonstrated by its ability to enhance collagen synthesis in UVB-treated HSF1184 fibroblast cells. The DPPH assay and HPLC analysis revealed a strong correlation ($r = 0.998$), indicating the freeze-dried extract has antioxidant content of 0.92 ± 0.02 mg AAE/g and 0.69 ± 0.06 mg AA/g, respectively. Collagen synthesis was enhanced by 92.86% and 143.65% at 100 µg/mL and 500 µg/mL of *C. caudatus* aqueous extract, comparable to the 97.85% enhancement observed with 100 µg/mL of AA. These findings highlight the potential of the freeze-dried aqueous extract of *C. caudatus* for further study in cosmeceutical research. Further research on formulation development, stability, skin permeation, and *in vivo* efficacy is needed before practical application.

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Declaration of Competing Interests

The authors declare no conflicts of interest.

CRedit Author Contribution Statement

S. Ibrahim: Methodology; Analysis; Investigation; Writing – original draft.
H. F. Ismail: Resources; Formal analysis; Writing – review & revise
R. Abu: Conceptualisation; Funding acquisition; Writing – review & revise; Supervision.

Availability of Data and Materials

Available on request: The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Generative Artificial Intelligence Disclosure

All intellectual content is original and solely attributable to the authors. The authors used AI-based tools solely for language editing and grammar refinement.

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