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# Biochemical and functional properties of extracts from *Cordyceps militaris* residues enriched with *Styphnolobium japonicum* flowers

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**Abstract.** Medicinal fungus *Cordyceps militaris* is widely cultivated in Vietnam, yet its solid-state cultivation residues are often discarded, representing a loss of potentially valuable bioactive compounds. In this study, the solid medium was supplemented with 50% *Styphnolobium japonicum* flower powder to investigate the effects of fermentation on biochemical composition and bioactivities of the fermented extracts. These extract maintained the fruiting body yield while exhibiting significantly enhanced antioxidant activity ($SC_{50}$ = 98 μg/mL), a 1.5-fold increase over the unfermented control. Meanwhile, the antimicrobial, anti-inflammatory, tyrosinase inhibitory, and cytotoxic activities against cell lines of the post-fermentation sample remained comparable with those of the pre-fermentation sample. Although the increase in total phenolic and total flavonoid content after fermentation was statistically insignificant, several biochemical changes were observed in the TLC and HPLC profiles of the post-fermentation sample compared with the pre-fermentation control. These results suggest that supplementing medicinal substrates with plant-derived powders can effectively enhance the functional properties of the fermented products. Furthermore, this approach provides a sustainable strategy for valorising underutilised solid residues, adding economic and environmental value to *C. militaris* cultivation. Overall, the study highlights the potential of integrating plant supplementation and solid-state fermentation as a practical method to produce bioactive-rich extracts, contributing to the development of functional foods, nutraceuticals, and other value-added products from medicinal fungi.

**Keywords:** *Cordyceps militaris*, medicinal plant, fermentation, *Styphnolobium japonicum*

## 1 Introduction

In recent years, the sustainable utilisation of agricultural and industrial by-products has emerged as a critical priority in both environmental management and bioresource innovation. Bioconversion processes, particularly those involving medicinal fungi, offer a promising approach to transforming low-value residues into high-value functional products.

*Cordyceps militaris*, a typical species of the genus *Cordyceps* (Hypocreales, Ascomycetes), is an entomopathogenic fungus that parasitises the larvae or pupae of lepidopteran insects. Similar to *C. sinesis*, *C. militaris* produces numerous bioactive ingredients and is used widely as traditional medicine and healthy foods [1]. Since the successful development of artificial cultivation techniques, *C. militaris* has become an economically prominent species because of its high medicinal value, market demand, and significant potential for economic growth. In Vietnam, its cultivation has expanded rapidly, and a wide range of products derived from this fungus are now accessible to consumers. However, the solid-state cultivation method—commonly employed for *C. militaris* production—generates large amounts of solid-based residues,

posing an environmental challenge that requires sustainable management.

*Styphnolobium japonicum* L. belongs to the Fabaceae family and is a ubiquitous plant in Vietnam, China, Japan, and Korea, and has long been used in traditional medicine to treat various conditions such as hemorrhoids, hematuria, hematochezia, hematemesis, uterine or intestinal hemorrhage, arteriosclerosis, headache, hemorrhinia, hypertension, dysentery, dizziness, and pyoderma. Modern pharmacological studies have revealed that its bioactive constituents and crude extracts possess diverse biological activities, including cardiovascular benefits, as well as anti-inflammatory, anti-osteoporotic, antioxidant, antitumor, antibacterial, antiviral, hemostatic, and anti-atherosclerotic effects [2].

In recent years, microbial bioconversion utilising bacteria and fungi has attracted considerable interest in enhancing plant-derived materials' biochemical and functional properties. Fermented plant extracts are rich in vitamins, minerals, and secondary metabolites that provide health-promoting effects, including intestinal cleansing, detoxification, anti-inflammatory, antibacterial, antioxidant, and antitumor activities [3]. *C. militaris* fermented leaf extract (*Morus alba*), *Panax ginseng*, and soy whey have been reported to increase biological activity and biochemical composition [4–6]. Thus, *C. militaris* is able to perform biotransformation of available compounds, enhancing or changing the biological activity of the fermented products. It has considerable potential for use in the food, chemical, and pharmaceutical industries.

This study aims to evaluate the biotransformation of *S. japonicum* flowers by *C. militaris* during solid-state fermentation to utilise solid-based residues, a source of agricultural waste effectively.

## 2 Materials and methods

### *C. militaris* culture and solid-based residue extraction

*C. militaris* was activated on a PDA medium. The solid-state medium for *C. militaris* stroma production was supplemented with *S. japonicum* flower powder (SFP) at different ratios (0, 12.5, 25, 50, and 100%) to the brown rice component. The *S. japonicum* powder was prepared by finely grinding dried *S. japonicum* flowers. *C. militaris* was cultured at different stages according to Kang et al. [7]. The experiments were designed with the following control samples: C-control: solid medium with *C. militaris*; R-control: solid medium without *C. militaris*; S-control: solid medium containing S. *japonicum* powder without *C. militaris*; CS sample: solid medium: *S. japonicum* powder with *C. militaris*. The solid-based residues were dried and extracted with 80% ethanol at a 1:10 (w/v) ratio for 2 hours under boiling. The extract was then evaporated with a rotary evaporator (IKA-Germany) at 30–40 °C.

### Biochemical analysis

The total phenolic content was determined via spectrophotometric analysis with Folin-Ciocalteu reagent [8]. The extracts were dissolved in 80% ethanol to obtain a stock solution (1 mg/mL), then serially diluted. One hundred microlitres of sample or standard substance were added to a 96-well plate, followed by 10 μL of Folin-Ciocalteu reagent. After 5 minutes, 100 μL of 7% $Na_2CO_3$ was added. The absorbance of the resulting blue solution was measured at 765 nm (Biotek, USA) after incubation at ambient temperature for 90 minutes. Gallic acid was used to generate the standard calibration curve. The total phenolic content was expressed as micrograms of gallic acid equivalents per milligram of dry extract (μg GAE/mg extract).

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The total flavonoid content was determined according to the method described by Choi et al. [9]. The extracts were dissolved in 80% ethanol to prepare a stock solution (5 mg/mL), followed by serial two-fold dilutions to obtain various concentrations. Quercetin standard solutions were prepared at 0, 50, 100, 200, and 300 μg/mL concentrations. Twenty microlitres of sample or standard solution were added to a 96-well plate, followed by 4 μL of 10% $Al(NO_3)_3$, 4 μL of 1 M potassium acetate, and 172 μL of 80% ethanol. The mixture was incubated at ambient temperature for 40 minutes. Absorbance was then measured at 415 nm with a spectrophotometer. A standard curve was constructed with quercetin, and the total flavonoid content of the extracts was calculated accordingly. Results were expressed as micrograms of quercetin equivalent per milligram of extract (μg QE/mg extract).

**Thin-layer chromatography analysis**

The extracts were dissolved in 80% ethanol to obtain a final concentration of 25 mg/mL. Pre-coated silica gel 60 $F_{254}$ plates were used for thin-layer chromatography (TLC). The solvent system consists of ethyl acetate, methanol, and water with a 6:1:1 ratio. The films were visualised under UV light at wavelengths of 365 nm and 254 nm.

**High-performance liquid chromatography (HPLC) analysis**

The extracts were dissolved in methanol at a 100 μg/mL concentration and analysed with a reversed-phase Mightysil RP-18 GP 250-4.6 (5 μm) column. The mobile phase consisted of water and acetonitrile, and the gradient elution program lasted over 30 minutes, as follows: acetonitrile content increased from 0 to 50% (0–18 min), from 50 to 100% (18–20 min), maintained at 100% (20–22 min), and returned to 0% (22–30 min). The detection was carried out at 254 nm with a flow rate of 1.0 mL/min [7].

**Bioactivity assays**

The extracts' antioxidant activity was evaluated as their DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity following the method described by Mohammad et al. with some modifications [10]. The extracts were dissolved in 80% ethanol to prepare a 10 mg/mL stock solution; then, they were serially diluted to obtain various test concentrations. Ascorbic acid was used as a positive control at concentrations ranging from 0 to 0.1 mg/mL. The DPPH solution (0.1 mM) was also prepared in 80% ethanol. Twenty microlitres of extract were mixed with 180 μL of the 0.1 mM DPPH solution in a 96-well plate and incubated in the dark at ambient temperature for 30 minutes. The absorbance was measured at 517 nm with a microplate spectrophotometer (Biotek, USA), and the $SC_{50}$ value (the concentration required to scavenge 50% of DPPH radicals) was determined.

The anti-inflammatory activity was evaluated as the sample's ability to inhibit nitric oxide (NO) production in RAW 264.7 macrophage cells according to Tsai et al. [11]. The extracts were dissolved in dimethyl sulfoxide to obtain final concentrations of 256, 64, 4, and 1 μg/mL. The $IC_{50}$ value, defined as the concentration required to inhibit 50% of NO production, was determined.

The broth dilution method was utilised to evaluate the antibacterial activity of the extract as the minimum inhibitory concentration (MIC). Resazurin, a colourimetric indicator, was employed to detect bacterial growth on the basis of colour change according to Ngan et al. [12]. Seven bacterial strains were tested, namely three Gram-positive strains (*Bacillus subtilis* ATCC 6633, *Micrococcus luteus*, and *Enterococcus faecalis* 19433), three Gram-negative strains (*Klebsiella pneumoniae* ATCC 10031, *Salmonella enterica* ATCC 14028, and *Escherichia coli* ATCC 25922), and *Staphylococcus aureus* CCARM 3634, MRSA, a methicillin-resistant strain. The extracts were diluted to

obtain 15, 10, 5, 2.5, and 1.25 mg/mL final concentrations in each well. The bacterial suspension was adjusted to a final concentration of $10^6$ CFU/mL in each well of the 96-well plate. Each treatment was performed in triplicate. After 24 hours, 20 μL of a 0.01% resazurin solution (blue) was added to each well. Colour change was observed, and the MIC was determined as the extract's lowest concentration that prevented any colour change of the resazurin indicator, demonstrating inhibition of bacterial growth.

The cytotoxic activities against cancer cell lines: KB, A549, HepG2, MCF7, and HEK293 were evaluated with the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, according to McCauley et al. [13]. The extracts were dissolved in dimethyl sulfoxide and tested across a of 256–1 μg/mL concentration range. Ellipticine (0.01 mM) was used as a positive control. Cytotoxic potential was assessed *in-vitro* by determining the $IC_{50}$ value, a concentration required to inhibit 50% of cell proliferation.

The *in-vitro* tyrosinase inhibitory activity of the extracts was evaluated according to Prommaban et al. [14]. The extracts were two-fold diluted in a 50 mM phosphate buffer (pH 6.5) to obtain concentrations ranging from 10 to 1.25 mg/mL. Tyrosinase (1000 U/mL) and L-DOPA (10 mM) were also prepared in the phosphate buffer. 20 μL of extract, 120 μL of phosphate buffer, and 20 μL of tyrosinase were added to a 96-well microplate and incubated at ambient temperature for 10 minutes, followed by the addition of 40 μL L-DOPA and a further 10-minute incubation. Kojic acid was used as a positive control. The absorbance was measured at 475 nm with a Microplate Reader (Biotek, USA).

**Statistical analysis**

All experiments were performed in triplicate, and data were expressed as mean ± SD. Statistical analysis was performed by using SPSS 26.0, with one-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons. A *p*-value of less than 0.05 was considered statistically significant.

## 3 Results and discussion

### 3.1 Effects of SFP on *C. militaris* yield

The results in Table 1 show that adding SFP to the rice-pupae medium insignificantly changed the fresh yield of the fruiting body. It can be seen that there is no statistical difference in the dry yield of the 12.5–50% SFP-supplemented samples. However, there was a decrease in the dry yield of these samples compared with the control, but in a concentration-independent manner. Therefore, the yield reduction may not be due to the secondary compounds in *S. japonicum* flowers. However, the SFP powder changed the solid medium's structure, thereby affecting fruiting body yield.

**Table 1.** Yield of *C. militaris* fruiting body

| | Fresh weight (g/bottle) | Dry weight (g/bottle) |
|---|---|---|
| 0% SFP | 37.08 ± 2.8[a] | 6.41 ± 0.3[a] |
| 12.5% SFP | 36.23 ± 1.8[a] | 5.57 ± 0.24[b] |
| 25% SFP | 33.32 ± 2.0[a] | 5.33 ± 0.14[b] |
| 50% SFP | 34.63 ± 2.2[a] | 5.0 ± 0.18[b] |
| 100% SFP | ND | ND |

*ND: Not detected. Letters in the same column indicate statistically significant differences ($p < 0.05$)*

Yao et al. demonstrated that essential oils extracted from the flower buds of *S. japonicum* did not inhibit the growth of *Aspergillus niger* and *Candida albicans* [15]. Similarly, Zhang et al. employed five different extraction solvents (water, ethanol, methanol, acetic acid, and ethyl acetate) and found that none of them showed

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inhibitory effects on *A. niger* or *Saccharomyces cerevisiae* [16].

Substrate permeability factors can influence mycelial spread and growth. Sofi et al. reported that different cereal grains used as substrates for *Pleurotus ostreatus* cultivation significantly affected the colony diameter [17]. Nguyen et al. showed that *Canna edulis* pomace waste in the *Pleurotus florida* mushroom culture medium increased yield compared with straw and cotton [18].

Although SFP supplementation failed to enhance the fruiting body yield, it aligns with the overarching goal of creating a dual-benefit cultivation system—producing medicinal mushrooms while simultaneously generating functional by-products.

### 3.2 Chemical compound contents

The results in Table 2 show that the high content of total phenolics and total flavonoids in the CS sample mainly resulted from SFP. No significant increase in the amount these components was observed between the pre-fermentation (S-control) and post-fermentation samples.

The results of this study are consistent with previous findings, revealing that *S. japonicum* flower buds had a high flavonoid content, reaching 151.86 QE mg/g extract [19]. Meanwhile, the total flavonoid content was low with *C. militaris* at 5.19 QE mg/g extract in the fruiting bodies and 7.64 QE mg/g extract in the mycelia [20].

Flavonoids and isoflavonoids are considered the main bioactive secondary metabolites of *S. japonicum*, especially rutin, a quercetin glycoside, accounting for 6–30% of its composition [21]. This compound may be a substrate for some fungal hydrolytic enzymes during fermentation, such as β-glucosidase, producing products that may have higher activity or react more strongly with the colour reagents in phenolic or flavonoid colour assays. Furthermore, hydrolytic enzymes also play a role in cell wall hydrolysis, leading to better release of secondary compounds, which may increase the extraction efficiency of the post-fermentation product. Similar results were observed in soy whey fermented with *C. militaris* [6] or kombucha [22], where phenolic and flavonoid contents increased after fermentation, suggesting that microorganisms can efficiently convert the isoflavone glucosides into their aglycone forms during fermentation.

**Table 2.** Total flavonoid content and phenolic content of solid-based residue extracts

| | **Total flavonoids (µg QE/ mg extract)** | **Total phenolics (µg GAE/mg extract)** |
|---|---|---|
| R-control | $1.62 \pm 0.36^{a}$ | $54.21 \pm 1.09^{a}$ |
| C-control | $7.82 \pm 0.48^{a}$ | $79.82 \pm 7.52^{a}$ |
| S-control | $181.63 \pm 6.04^{b}$ | $206.88 \pm 9.31^{b}$ |
| CS sample | $202.26 \pm 6.72^{b}$ | $251.02 \pm 12.52^{b}$ |

*Note:* CS sample: Extract from residues of *C. militaris* cultured with 50% SFP; S-control: Extract from medium with 50% SFP, no *C. militaris*; C-control: Extract from residues of *C. militaris* cultured without SFP; R-control: Extract from medium without *C. militaris* and SFP. Letters in the same column indicate statistically significant differences ($p < 0.05$)

Thin-layer chromatography and high-performance liquid chromatography were employed to analyse the extracts. The results in Fig. 1 and Fig. 2 revealed the presence of several new spots (Fig. 1) and new peaks (Fig. 2) compared with the control samples. This indicates that *C. militaris* was involved in the biotransformation of certain compounds in the SFP material during its growth. Peak No. 13 in the CS sample (Fig. 2) corresponds to the rutin standard peak, showing a decrease in intensity compared with the S-control sample (non-fermented *S. japonicum* powder control).

However, no quercetin peaks were observed in the CS sample. Thus, the decrease in rutin may have other reasons than the hydrolysis of the sugar moiety (rutinoside) to quercetin.

The application of *Cordyceps* species, especially *C. sinensis* and *C. militaris*, in fermenting plant materials to enhancing bioactivity has been extensively reported, with accompanying chemical transformations. Wang et al. showed that fermenting the *Panax ginseng* extract with *C. sinensis* converted ginsenoside Rb1 into compound K with more potent antitumor effects [23]. Mira et al. reported that fermentation of *Angelica shikokiana* extract with *C. sinensis* modified isoepoxypteryxin, increasing cytotoxicity and introducing antiplatelet aggregation activity [24]. Li et al. found that fermenting ginseng with *C. militaris* boosted cordycepin, polysaccharide, and pseudoginsenoside F11 production, highlighting applications in cosmetics and pharmaceuticals [5]. More recently, Dai et al. demonstrated that soy milk fermented with *C. militaris* had higher amino acid, phenolic, flavonoid, and aglycone isoflavone contents, reduced indigestible oligosaccharides, enhanced ABTS radical scavenging, and improved DNA protection [6].

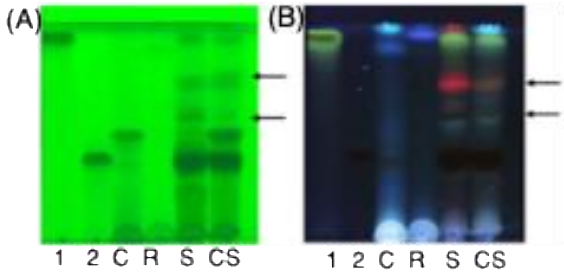


**Fig. 1.** Thin-layer chromatography analysis of the extracts under UV light at 254 nm **(A)** and 365 nm **(B)**. *1*: quercetin; *2*: rutin (reference standards), C-control: solid medium with *C. militaris*; R-control: solid medium without *C. militaris*; S-control: solid medium containing *S. japonicum* powder without *C. militaris*; and CS sample: solid medium containing *S. japonicum* powder with *C. militaris*

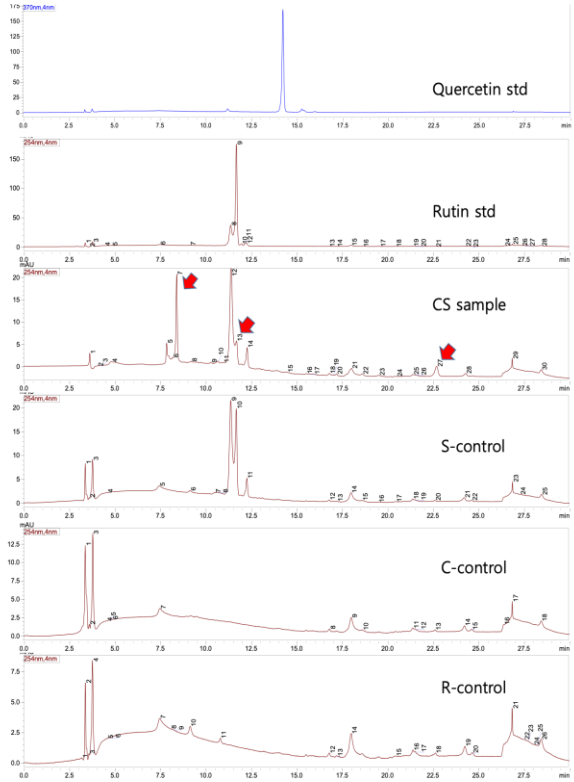


**Fig. 2.** HPLC chromatography of solid-based residue extracts. The red arrow points to peaks that are different from those of the control samples

These findings suggested that the fermentation of medicinal plant extracts with fungi represents a promising approach to enhancing the bioactivity of both the fungal cultures and the herbal substrates.

### 3.3 Antioxidant activity

The results of the antioxidant activity of the extract samples (Fig. 3) showed that the CS sample had powerful antioxidant activity compared with the control samples.

The results demonstrated a statistically significant increase in antioxidant activity of all fungal-cultivated substrates compared with the non-inoculated control. Notably, the formulation containing SFP and inoculated with *C. militaris* (CS sample) exhibited antioxidant activity six-fold that of the C-control and 1.5-fold the R-control

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with $SC_{50}$ values of 0.098, 0.595, and 0.147 mg/mL, respectively.

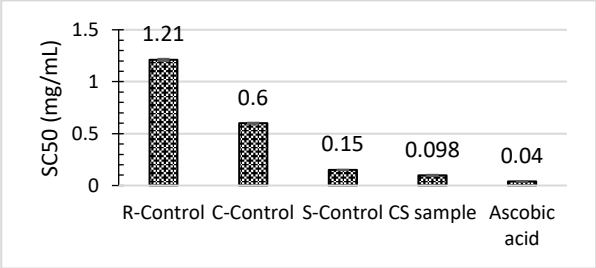


**Fig. 3.** 50% Scavenging capacity of solid-based residue extracts (*Letters above column indicate statistically significant differences ($p < 0.05$).*)

The higher antioxidant activity in the CS sample may result from *C. militaris* bioactive compounds and fermentation-induced transformations of SFP biochemicals.

The significant difference between the C-control and R-control samples showed that the contribution of *C. militaris* to antioxidant activity was prominent. The antioxidant activity of *C. militaris* is associated with its polysaccharide and cordycepin content [25]. It has been shown that *C. militaris* possesses antioxidant activity, as evidenced by DPPH radical scavenging with an $EC_{50}$ of 12.17 mg/mL [26].

As discussed above, although phenolic and flavonoid contents increase slightly, fermentation induces the transformation of certain compounds in SFP. Flavonoids, the main constituents of *S. japonicum* flowers, such as rutin, may have been converted to the aglycone quercetin. These compounds likely contribute to the antioxidant activity observed in the CS extract. The antioxidant properties of flavonoids and isoflavones from *Fructus sophorae* [27] and rutin from *Flos sophorae immaturus* [28] have been previously reported. These findings suggest that the notable antioxidant activity of *S. japonicum* is closely associated with its key flavonoids, particularly rutin and quercetin.

### 3.4 Antibacterial activity

The antibacterial activity of the extracts was evaluated against seven bacterial strains: four Gram-positive and three Gram-negative strains. The CS sample extract exhibited activity against *M. luteus* with a MIC value of 15 mg/mL. Meanwhile, the MIC value of the CS sample could not be established for the other strains at the tested concentration of 15 mg/mL. The control samples without *C. militaris* (R-control and S-control) did not show antibacterial activity against all seven bacterial strains at the tested concentrations.

Notably, the C-control extract exhibited better antibacterial activity among the control samples. It effectively inhibited *S. enterica* and *M. luteus* with a MIC of 15 mg/mL and showed strong activity against Methicillin-resistant *Staphylococcus aureus* (MRSA), with a MIC of 10 mg/mL.

This result was consistent with that of previous studies regarding the antibacterial properties of *C. militaris*. Methanolic extracts of *C. militaris* have demonstrated potent antibacterial activity against *Bacillus cereus* and *Pseudomonas aeruginosa* with particularly low MIC and MBC values (0.015 and 0.03 mg/mL) [26].

A study by Kimura and Yamada demonstrated that ethanol extracts of *S. japonicum* flowers featured notable antibacterial activity against *Propionibacterium* species, bacteria implicated in acne inflammation. The extract significantly inhibited *P. acnes, P. avidum*, and *S. aureus* under mildly acidic conditions. This effect was attributed to the interaction among three flavonoids: quercetin, rutin, and isorhamnetin-3-rutinoside. Quercetin exhibited only weak activity when tested individually, while the other compounds were inactive. However, combining quercetin with either rutin or isorhamnetin-3-rutinoside restores 50–70% of the extract's

antibacterial potency, and the combination of all three fully recovered the original activity [29]. Therefore, the activity of *S. japonicum* flower extract might depend on the presence of these three substances and their relative concentrations. In our study, both pre- and post-fermentation SFP samples did not show antibacterial activity at the tested concentrations.

### 3.5 Anti-inflammatory activity

As presented in Table 3, regarding anti-inflammatory activity, the CS extract showed moderate inhibition with an $IC_{50}$ value of 192 μg/mL. In comparison, the control samples (R-control and S-control) displayed comparable $IC_{50}$ values ranging from 186.18 μg/mL (C-control) to 226.46 μg/mL (S-control), with no statistically significant differences among them.

The anti-inflammatory activity of *C. militaris* resulted from its ability to suppress the production of pro-inflammatory mediators, specifically NO, TNF-$\alpha$, and IL-6 [30]. Regarding *S. japonicum*, its anti-inflammatory effects are mainly attributed to its chemical constituents, particularly rutin. This compound has been shown to improve the morphology of RAW264.7 cells stimulated by lipopolysaccharide, thereby effectively reducing the inflammatory response [31].

**Table 3.** $IC_{50}$ values of solid-based residue extracts and reference compound

| Samples | $IC_{50}$ (μg/mL) |
|---|---|
| R-control | >256 |
| C-control | $186.18 \pm 5.62^{a}$ |
| S-control | $226.46 \pm 8.9^{a}$ |
| CS sample | $192.00 \pm 6.54^{a}$ |
| L – NMMA | $11.00 \pm 0.80^{b}$ |

*Letters in the column indicate statistically significant differences ($p < 0.05$).*

### 3.6 *In-vitro* cytotoxic activity and tyrosinase inhibitory activity

The cytotoxic activity of the extracts was tested on cell lines KB, A549, HepG2, MCF7, and HEK293. The results showed that none of the four extracts exhibited cytotoxic activity at the maximum tested concentration of 256 μg/mL. Therefore, further evaluation at higher concentrations is necessary to clarify their potential anticancer effects.

A previous study showed that *C. militaris* cultivated in a medium supplemented with *Radix astragali* could enhance its anticancer activity. The resulting fermented extract inhibited the proliferation of AGS, MCF-7, Hep G2, and CT26 cancer cells, with the most potent effects observed at 25 °C, showing $IC_{50}$ values of 465.17, 36.9, 24.63, and 20.28 μg/mL, respectively. Although the cordycepin content was lower in the *R. astragali*-supplemented medium, its anticancer activity was higher than that of the synthetic medium [32].

The tyrosinase inhibitory activity of *S. japonicum* flower bud extracts at different stages was reported by Wang et al. They found that the ES3 (bud cracking) stage showed the highest inhibition (38.27%), although rutin and total flavonoid contents were higher in earlier stages. This suggested that quercetin, which peaked in ES3, played a more critical role than rutin in tyrosinase inhibition [33].

Pintathong et al. also found that the extracts from *C. militaris*-based residues on different types of rice exhibited tyrosinase inhibitory effects. The Riceberry-based substrate showed the highest value of 51.13 mg KAE (kojic acid equivalent)/g, with inhibition rates ranging from 75.76 to 90.43% at 1 mg/mL [34]. Additionally, cordycepin—a major bioactive compound in *C. militaris*—demonstrated inhibitory effects on melanin and tyrosinase in B16F10 melanoma cells, reaching 51.4% inhibition at 0.25 mg/mL [5].

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The results of our study were similar to those of previous reports, reporting that the tyrosinase inhibitory activity of the C-control sample had an $IC_{50}$ value of 7.6 mg/mL. No inhibitory activity was observed in the other extracts at the maximum tested concentration of 10 mg/mL.

## 4 Conclusion

This study demonstrates that supplementing *C. militaris* solid-state cultures with 50% SFP effectively enhanced the antioxidant activity of the resulting extracts, achieving a 1.5-fold decrease in $SC_{50}$ compared with the unfermented control. Fermentation also produced distinct chemical transformations, as confirmed by TLC and HPLC profiles. The extracts showed no cytotoxicity on human cell lines at the tested concentration. These findings support the potential of this approach to valorise *C. militaris* solid-based residues.

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