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STUDY OF THE CHEMICAL COMPOSITION AND BIOACTIVITY OF TEA BAGS FROM *Cordyceps militaris, Siraitia grosvenorii* AND *Chrysanthemum sinense*

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ABSTRACT

This study conducted a trial to mix three traditional medicinal herbs to make tea bags, such as *Cordyceps militaris*, *Siraitia grosvenorii*, and *Chrysanthemum sinense*. It investigated the chemical composition as well as the biological activity of the product. *Cordyceps militaris, Siraitia grosvenorii, and Chrysanthemum sinense* are precious medicinal species widely used in East Asian countries to make medicines and herbal b everages. However, research on the effects of tea bags with the above 3 ingredients has not yet been published in Vietnam. This study focuses on investigating the chemical composition, antioxidant, and anti-cancer activities of this tea bag to orient towards its application in food. The results of the study showed that the ratio of mixing *Cordyceps militaris:Siraitia grosvenorii:Chrysanthemum sinense* (w:w:w) of 2:1:1 obtained the highest sensory value and the basic chemical composition of the tea bag product was determined, including protein of 4%, the concentrations of polysaccharide of 23.4%, total polyphenol content of 56.34 mg GAE/g extract, adenosine of 0.183 mg/g extract, and cordycepin of 0.231 mg/g extract. An extract of the tea bag exhibited a free radical scavenging activity of DPPH with IC_{50} 428.4 µg/mL. The concentration of the extract of the tea bag up to 1600 ppm induced cell death with 49.63% of A549 cells and 62.82% of MCF7 cells. In addition to showing the advantages of a tea bag product from *Cordyceps militaris* combined with *Siraitia grosvenorii* and *Chrysanthemum sinense*, this study has explored new research directions with potential applications in food for the first time.

Keywords: Chrysanthemum sinense, *Cordycep militaris*, DPPH, MTT, *Siraitia grosvenorii*, tea bag.

1. INTRODUCTION

Herbal teas are widely consumed worldwide and play a significant role as a therapeutic agent in various traditional medical practices [1]. Several widely consumed herbal teas include black tea, green tea, chamomile tea, ginger tea, ginseng tea, peppermint tea, and cinnamon tea. Among these, certain varieties exhibit potent medicinal properties, such as Astragalus tea, derived from a Chinese native herb known for its anti-inflammatory and antibacterial effects [2]. The improvement in socio-economic status has seen an increase in demand for tea bags due to convenience, and ease of throwing away-cutting and blending many ingredients can be done in a small tea bag [3]. The quality of tea bags is primarily determined by the type of raw materials used, whether they consist of tea, herbs, or a combination of both.

Cordyceps militaris has a long history of use in traditional Chinese medicine [4]. *Cordyceps militaris* has been widely distributed in many parts of the world, but the largest concentration is still in East Asian countries such as China, Japan, and Korea [5]. Many studies have proven that *Cordyceps militaris* contains many kinds of bioactive components such as cordycepin, adenosine, and some polysaccharides. These compounds are effective in supporting and treating many diseases such as anti-inflammatory, antioxidant, anticancer, and immunomodulatory as well as the enhancement of liver, kidney, and lung function [6-9].

Siraitia grosvenorii is a perennial plant that cultivated mainly in Guangxi province of China for over 200 years [10]. *Siraitia grosvenorii* is rich in triterpenoids, flavonoids, vitamins, proteins, saccharides, and essential oils [11]. Mogrosides, a group of triterpenoid glycosides isolated from the fruit of *Siraitia grosvenorii*, are considered the main active substances for sweetness as well as are responsible for the major biological effects of *Siraitia grosvenorii* [12]. *Siraitia grosvenorii* fruit has been used for many centuries in China as a natural sweetener and a traditional medicine to treat lung congestion, colds, and sore throats [13]. Nowadays, the fruit *Siraitia grosvenorii* has been proven many benefits such as antitussive, anti-asthmatic, antioxidant, hepatoprotective, hypoglycemic, immunomodulatory, and anticancer [14].

Chrysanthemum sinense is a traditional medicinal species in Vietnam and a native plant of China [15]. The chemical composition of *Chrysanthemum sinense* includes flavonoids such as luteolin, cosmosin, acacetin-rhamnosin, and apigenin [16] and triterpene alcohols such as heliaol, lupeol, taraxerol, and cycloartenol. Stachydrine is a kind of compound of alkaloid that has also been found in *Chrysanthemum sinense*. In addition, *Chrysanthemum sinense* also contains amino acids such as choline, adenine, and some vitamins such as vitamins B1, C, E, and A [17]. In traditional Chinese medicines, *Chrysanthemum sinense* has been used as an herb with many beneficial effects such as anti-inflammatory, analgesic, antipyretic, and eye diseases [18, 19]. According to recent research, *Chrysanthemum sinense* inhibited the aggregation of platelets and promoted the circulation of myocardial blood [20].

Tea bags have many natural ingredients, conveniences, the treatment of many diseases, and the improvement of human health. Currently, there are many different types of tea bags (*Panax notoginseng* tea, lotus tea, etc.) in the Vietnamese market, but the combination of these three herbs has not been studied yet. Therefore, this study was conducted to provide information that would be the foundation of application in food.

2. MATERIALS AND METHODS

2.1. Materials

Cordyceps militaris was provided by the Institute of Tropical Biology. Fruiting bodies of *Cordyceps militaris* were oven-dried at 50-60 °C for 6-8 h until the moisture content was less than 10%. *Siraitia grosvenorii* and *Chrysanthemum sinense* were purchased at An Quoc Thai Traditional Medicine Pharmacy (Address: 347 Tran Hung Dao B, Ward 10, District 5, HCMC).

Chemicals: PBS (Phosphate buffered saline), pH 7.4, Himedia, India; DPPH (2,2-Diphenyl-1-picrylhydrazyl (free radical), 95%), Sigma-Aldrich, USA; MTT (3-(4,5-dimethyl-2-thiazolyl)-2-5-diphenyl-2*H*-tetrazolium bromide), Merck, Germany; acid ascorbic 99,75%, Duchefa, Netherlands; Na_2CO_3 99.8%; ethanol 96%; methanol 96%; acid hydrochloric (36% HCl); acid sulfuric (36% H_2SO_4); NaOH 99%; $FeCl_3$ 95%; $K_3[Fe(CN)_2]$ 99,5%; ethyl acetate 99%; sodium acetate buffer, pH 5.5, Sigma-Aldrich, USA.

2.2. Methods

2.2.1. Mixing ratio

Cordyceps militaris, Siraitia grosvenorii, and *Chrysanthemum* have been used as an herb to improve human health and to treat many diseases in the world. In Vietnam, dried *Cordyceps militaris* is used as a healthcare beverage. However, for some people, this usage has a slightly pungent taste that makes it difficult to drink. Therefore, the addition of fragrance and natural sweeteners from *Siraitia grosvenorii* and *Chrysanthemum* can enhance the attractive flavor of *Cordyceps militaris*. Dried *Cordyceps militaris, Siraitia grosvenorii,* and *Chrysanthemum* were ground into a fine powder (< 1 mm). The mixing of auxiliary materials with prices many times lower than *Cordyceps militaris* also helps to reduce the costs of the product and to reach consumers effectively. The basic samples were mixed in the ratios (w:w:w) of 1:1:1, 2:1:1, and 3:1:1 respectively, with 150 g of each sample and 3 replicates.

Each tea bag contained approximately 2 g of the blended samples. The material of the tea bag was food grade non-woven material, safe and hygienic, easy to break down, and environmentally friendly. Its variant was 6 cm × 8 cm. The tea bags were placed in tightly sealed glass bottles and appropriately labeled for analysis.

2.2.2. Extraction

After mixing at the above ratios, 10 g of ingredients (corresponding to 5 tea bags) into a 250 mL Erlenmeyer flask, added 100 mL of 80 °C hot water, and soaked for 24 hours at room temperature. Then, the extract was subsequently filtered using Whatman filter paper and lyophilized at a temperature of -70 °C. The crude extract obtained was stored at -20 °C in the dark until used to determine the chemical composition of tea bags.

2.2.3. Sensory evaluation

According to Vietnam's national standard method (TCVN 3215-79), samples were evaluated periodically for their sensory characteristics of structure, color, smell, and taste. The evaluation was based on a score scale rated in the interval from 1.0 to 5.0 for 4 parameters: structure, color, smell, and taste (Table 1), important coefficient was rated from 0.6 to 1.2 to express the important level of each parameter [21].

*Table 1***.** Important coefficient of each target

Quota	Important coefficient
Structure	1.0
Color	0.6
Smell	1.2
Taste	1.2

The total scores of each parameter were to multiply the score of each parameter and its important coefficient. The last sensory score of the product was the total score of the 4 parameters. If the last sensory score is 20, the sample meets the highest quality (Table 2).

Table 2. Assessing the quality of tea bag

Quality level	Common score	Quality level	Common score
Good type	18.6-20.0	Poor type	7.2-11.1
Pretty good type	15.2-18.5	Very poor type	4.0-7.1
Medium type	11.2-15.1	Type of damage	0.0-3.9

Notes when evaluating the sensory quality of products: Place the teacup in a balanced position and a well-lit room and keep your eyes on the right line to observe the color and status of the product. Hold the tea cup close to the nose, open the lid with the hands, and smell the product on the top of the cup to evaluate the smell. Taste the product in the mouth to determine its taste.

Conditions for conducting sensory evaluation: The sensory evaluation was done in a laboratory that had a quiet, cool, well-lit, and clean place. The people selected for sensory evaluation are those who have been trained and practiced in sensory evaluation of products and had good health such as specialist teachers, and students of food processing technology.

The number of testers selected for each experiment was 7.

2.2.4. Phytochemical analysis

The organic substances in the tea bag extracts were determined by standardized laboratory quantitative methods.

Samples were sent to the Center of Analytical Services and Experimentation of HCM City (CASE) to determine the content of the main ingredients in the product (adenosine and cordycepin) by the high-performance liquid chromatography (HPLC) method. After that, the determination of total protein content by the Kjendah method, and the determination of fat content by the Soxhlet machine.

The total phenolic content was quantified using the Folin-Ciocalteu assay, following the method outlined by Prieto et al. The crude extract was prepared in a series of dilutions with distilled water at varying concentrations. A 100 μL aliquot of the water-diluted extract was thoroughly mixed with 500 μL of Folin-Ciocalteu reagent for 4-5 minutes, followed by the addition of 400 μL of a 7.5% sodium carbonate solution. The mixture was incubated in the dark for 60 minutes, after which it was centrifuged and transferred into a 96-well plate. The absorbance at 765 nm was recorded using a microplate reader. A standard curve was generated using gallic acid (0.01-0.4 mM), and the results were reported as gallic acid equivalents (GAE) in mg/g.

Polysaccharide was quantified using the phenol-sunfuric acid method [22]. A 400 µL aliquot of the sample solution containing polysaccharides was thoroughly mixed with 200 µL of a 5% phenol solution, followed by the addition of 1 mL of concentrated sulfuric acid (H_2SO_4). The mixture was allowed to stand for a further at room temperature. The color of the reaction was detected on a spectrophotometer at 490 nm. The polysaccharide content was quantified based on the optical density obtained from the test sample against the glucose standard graph. The relative percentage value is the polysaccharide content of the highest group specified as 100%, and the other groups with the lower polysaccharide content as a percentage of the highest group.

2.2.5. DPPH assay

The antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, which measures free radical scavenging capacity. The total methanolic extract and its fractions were dissolved in ethanol at serial concentrations. Reaction mixtures, comprising 100 µL of the sample and 100 µL of DPPH solution, were prepared in a 96-well plate. After 30 minutes, the absorbance of the solution was measured at 517 nm. The DPPH radical scavenging activity was determined using the following equation:

$$\%\ \text{Scavenging} = \left[\frac{A\ (\text{control}) - A\ (sample)}{\text{A (control)}}\right] * 100$$

Each sample was mesured in triplicaite. The 50% inhibitory concentration (IC_{50}), defined as the concentration of the sample required to scavenge 50% of DPPH radicals, was calculated using probit-graphic interpolation across seven concentration levels. The commercial antioxidant (vitamin C 99.75% solution, Duchefa, Cas 50-81-7) was used as a positive control [23].

2.2.6. MTT assay

The tea bag extract was evaluated for its cell growth inhibitory effects on MCF7 and A549 cell lines using the MTT assay, as described by Dantu et al. (2012) [24]. MCF7 and A549 cell lines (provided by the Biotechnology Center, Ho Chi Minh City) were seeded in 96-well plates at a density of 40,000 cells per well. The cells were treated with the sample solution at varying concentrations and incubated for 72 hours. Subsequently, MTT solution (5 mg/mL) was added, and the cells were further incubated at 37°C for 4 hours. Afterward, the supernatants were removed, and 100 μL of isopropanol-HCl was added to each well to dissolve the formazan crystals. The optical density was determined at 540 nm. The cytotoxicity of each compound is expressed as an IC_{50} value.

2.2.7. Analysis of data

The experiment was repeated three times independently. Data values are expressed as mean ± SD (standard derivation). The IC50 value was obtained by plotting percentage inhibition (I%) versus concentration of total extract and tea bag extracts.

3. RESULTS AND DISCUSSION

3.1. Effects of mixed ratios

The results presented in Figure 1 showed that tea bags with a ratio of 2:1:1 gave the highest sensory score. According to TCVN 3215-79, this sample was classified as a pretty good type because it had an average total score of 16.63. Therefore, the ratio of 2:1:1 was selected for the subsequent experiments.

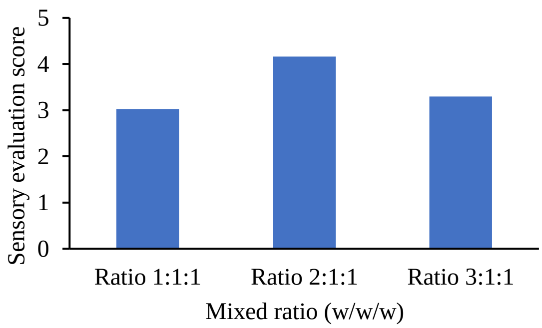

Figure 1. Effect of mixing ratio *Cordyceps militaris/Siraitia grosvenorii/Chrysanthemum* on sample quality

3.2. Chemical composition of tea bags

The Folin-Ciocalteu assay is one of the earliest methods established for determining the total phenolic content. A standard curve of gallic acid was built by preparing solutions of gallic acid with concentrations of 0, 25, 50, 100, 150, and 200 g/mL. The standard curve is shown in Figure 2. The total polyphenol content of the extract was quantified using a gallic acid standard curve and expressed as milligrams of gallic acid equivalents (GAE)/g extract. The content of total polyphenol in the extract of the tea bag sample was 56.31 mg GAE/g extract.

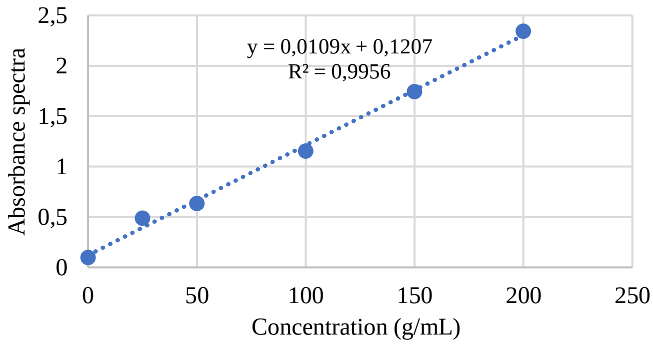

Figure 2. Standard curve of gallic acid

The results presented in Table 3 show that tea bags have a high polysaccharide content of 23.4%. In addition, the composition of the sample had a small amount of protein (4%), and fat was not detected. Polysaccharide was able to reverse the damage of ethanol-induced liver and this effect was enhanced with increasing doses of the extract. Yan et al. (2008), suggested that this effect may be due to the antioxidant function of polysaccharides from *Cordyceps militaris* [26]. Adenosine and cordycepin are two highly bioactive compounds of *Cordyceps militaris*. The content of adenosine and cordycepin in the tea bag sample is 0.183 mg/g extract and 0.231 mg/g extract, respectively.

Table 3. Chemical composition of tea bags

Compositions	Rate, %
Lipid	< MLQ* = 5%
Protein	4%
Polysaccharide	23.4%
Polyphenol	56.34 mg GAE/ g extract
Adenosine	0.183 mg/g extract
Cordycepin	0.231 mg/g extract

(*) Method Quantification Limit

3.3. Investigation of antioxidant activity of tea bags

The tea bag extract was evaporated into the extract form and diluted into an appropriate dilution at a range of different concentrations (0.039, 0.0781, 0.15625, 0.3125, 0.625, 1.25 mg/mL). The total antioxidant potential of tea bags was determined by using the DPPH assay. The antioxidant activities of tea bags are presented in Figure 3. According to our results, tea bags had a high antioxidant activity with IC_{50} = 428.4 µg/mL. This study showed the antioxidant capacity of cordycepin contained in the extract obtained from the culture of *Cordyceps militaris*. The following research by Zhang et al. (2013) reported cordycepin concentrations of 0.8 and 1.8 mg/mL, respectively [27]. An antioxidant capacity to remove OH and DPPH free radicals can reach 50%. This result is consistent with the cordycepin concentration in the extract we obtained. Zhang's research suggests that cordycepin's antioxidant properties are near to that of ascorbic acid. Thus, cordycepin in tea bag products has an antioxidant capacity (IC_{50} = 428.4 µg/mL). Our research has demonstrated potential with materials of HTLC originating from Vietnam.

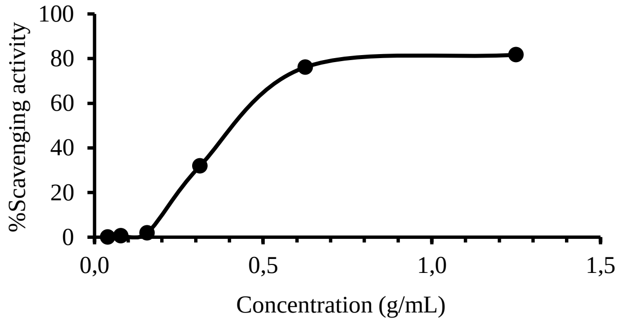

Figure 3. Antioxidant activity of tea bag extract using DPPH test.

3.4. Investigate of Anticancer effect of tea bags in human cancer cell lines A549 and MCF7

For the investigation of the toxicity of tea bags, cancer cell lines A549 and MCF7 were pretreated with total tea bag extracts at different concentrations (from 50 ppm to 1600 ppm for 72 h). Toxicity was determined by the MTT assay. The results showed that the concentration of tea bags up to 1600 ppm can inhibit 49.63% of A549 cells and 62.82% of MCF7 cells.

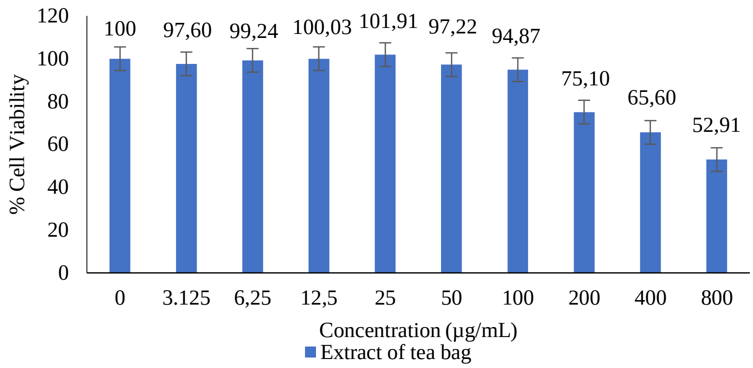

Figure 4. Effect of total tea bag extract on lung cancer (A549) cell line

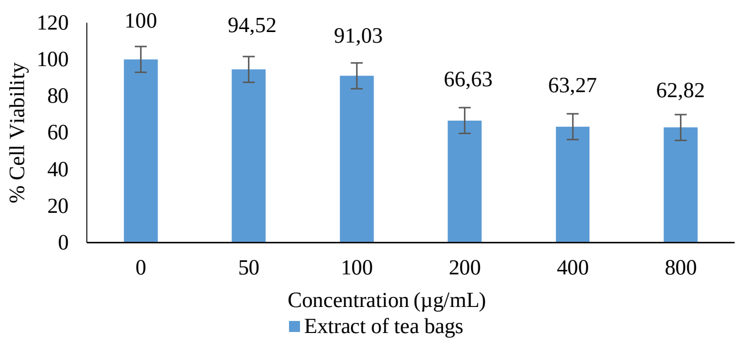

Figure 5. Effect of total tea bag extract on breast cancer (MCF7) cell lines

The results in Figure 4 show that the cordycepin sample in tea bag extract exhibited cytotoxic activity in both lung cancer (A549) and breast cancer (MCF7) cell lines at concentrations of 50, 100, 200, 400, and 800 ppm. The graph of Figure 5 clearly shows that the cytotoxic potential of cordycepin compounds in the extract is directly proportional to the concentration of the analyte. Therefore, increased cordycepin concentration corresponds to a greater ability to inhibit cell proliferation.

4. CONCLUSIONS

The combination of the ratio of *Cordyceps militaris, Siraitia grosvenorii,* and *Chrysanthemum* (2:1:1) has created a tea bag sample that has a high sensory value. The extract of the tea bag sample has the content of total polyphenol and polysaccharide with 56.31 mg GAE/g extract and 23.4% w/v extract. The high amount of adenosine and cordycepin in the tea bag is 0.183 mg/g extract and 0.231 mg/g extract, respectively. The extract of the tea bag has the free radical scavenging activity of DPPH with IC_{50} 428.4 µg/mL and induces cell death with 49.63% of A549 cells and 62.82% of MCF7 cells at a concentration of 1600 ppm. The present study highlights that the tea bag with the combination of *Cordyceps sinensis*, *Siraitia grosvenorii,* and *Chrysanthemum* has the potential for use in food applications.

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TÓM TẮT

KHẢO SÁT THÀNH PHẦN HÓA HỌC VÀ HOẠT TÍNH SINH HỌC CỦA TRÀ TÚI LỌC TỪ ĐÔNG TRÙNG HẠ THẢO (*Cordyceps militaris*), LA HÁN QUẢ (*Siraitia grosvenorii*) VÀ HOA CÚC (*Chrysanthemum sinense*)

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Nghiên cứu này tiến hành thử nghiệm phối trộn ba loại dược liệu truyền thống là Đông trùng Hạ thảo (*Cordyceps militaris*), La hán quả (*Siraitia grosvenorii*) và Hoa cúc (*Chrysanthemum sinense*) để chế biến sản phẩm trà túi lọc, đồng thời khảo sát thành phần hóa học cũng như hoạt tính sinh học của sản phẩm. Đông trùng Hạ thảo, La hán quả và Hoa cúc là các loài dược liệu quý, được sử dụng rộng rãi tại các nước Đông Á để làm thuốc và làm đồ uống. Tuy nhiên, nghiên cứu về tác dụng của trà túi lọc với 3 thành phần trên vẫn chưa được công bố ở Việt Nam. Nghiên cứu này tập trung khảo sát thành phần hóa học và hoạt tính kháng oxy hóa, kháng ung thư của trà túi lọc này, định hướng ứng dụng trong thực phẩm. Kết quả nghiên cứu cho thấy, tỷ lệ phối trộn *Cordyceps militaris:Siraitia grosvenorii:Chrysanthemum sinense* là 2:1:1 cho giá trị cảm quan cao nhất và hàm lượng tính theo khối lượng dịch chiết lần lượt gồm protein 4%, polysaccharide 23,4%, polyphenol tổng số 56,34 mg GAE/g, adenosine 0,183 mg/g và cordycepin 0,231 mg/g. Hoạt tính kháng oxy hóa thể thiện qua hiệu quả loại bỏ gốc tự do DPPH của dịch chiết trà túi lọc đạt IC_{50} = 428,4 µg/mL. Thử nghiệm độc tính trên các dòng tế bào cho thấy ở nồng độ 1600 ppm dịch chiết trà túi lọc gây chết 49,63% tế bào A549 và 62,82% tế bào MCF7. Nghiên cứu này, lần đầu tiên cho thấy những ưu điểm của sản phẩm trà túi lọc từ Đông trùng Hạ thảo kết hợp với La hán quả và Hoa cúc, đồng thời mở ra hướng nghiên cứu mới có tiềm năng ứng dụng trong thực phẩm.

Từ khóa: Chrysanthemum sinense, *Cordycep militaris*, DPPH, MTT, *Siraitia grosvenorii*, trà túi lọc.