

PHYTOCHEMICAL SCREENING AND MINERAL ELEMENTS COMPOSITION OF
CORDYCEPS SINENSIS AND ITS BASED PRODUCT (ESULIN)

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ABSTRACT: Screening of phytochemical and mineral elements composition of *Cordyceps sinensis* and its based product, Esulin were carried out in order to provide a basis for further research on the therapeutic value of these herbs. The results of both aqueous extracts positively showed the presence of several bioactive compounds like alkaloids, saponins, tannins, and flavonoids. However, terpenoids only present in the aqueous extract of Esulin. In addition, the levels of copper, zinc, cadmium, ferum, lead and nickel of both extracts were below the maximum permissible level suggested by World Health Organization (1989) & Malaysian Food Law (1985). The presence of phytochemical components and mineral elements in the aqueous extract of *Cordyceps sinensis* and Esulin might contribute to their therapeutic applications in medicinal practices.

Key words: Phytochemicals, mineral elements, *Cordyceps sinensis*, Esulin

INTRODUCTION

Herbal remedies have been used throughout the world over 4000 years ago for treatment of various diseases due to the presence of beneficial chemical elements in them. In essence, medicinal potentials of plants lie in their phytochemical constituents that produce a definite pharmacological action when applied to the human body. Phytochemicals are naturally occurring in medicinal plants, leaves, vegetables and fruits that have their own defence mechanisms and protection ions from various diseases (Wadood *et al.*, 2013). Among the important bioactive phytochemicals including alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952). Due to the effectiveness in treating various ailments, *Cordyceps sinensis* is frequently chosen and world widely used as an old folk therapeutic agent. *C. sinensis* is classified as an Ascomycetes fungus closely related to mushrooms (Halpern, 2007). Although not actually a mushroom taxonomically, it has been regarded as an exotic medicinal mushroom and has been appreciated for many centuries in traditional Chinese and Tibetan medicines (Zhu *et al.*, 1998). *C. sinensis* is the composite of a genus of fungus that grows on the larvae of *Hepialus armoricanus Oberthur* and therefore also known as caterpillar fungus. In nature, it grows only at high altitude regions of about 3800-4000m above sea level, in cold, grassy, alpine meadows of the Himalayan mountains and thus difficult to harvest. Because of this, *C. sinensis* is one of the most expensive medicinal herbs in the world (Kobayasi, 1982; Bhandari *et al.*, 2010). This fungus grows annually and its normal harvesting period is between April and August every year (Kobayasi, 1982). In scientific research, *C. sinensis* has been claimed to possess several pharmacological functions such as enhance physical stamina and increase energy by improving utilization of oxygen (Dai *et al.*, 2001). On other aspect, *C. sinensis* has been claimed to improve kidney function and reduce the damage caused by nephrotoxic chemicals (Li *et al.*, 1996). This fungus also has been reported could improve heart function in treating heart rhythm disturbances such as cardiac arrhythmia and chronic heart failure. The presence of adenosine as one of the bioactive ingredients in *C. sinensis* has been suggested to exhibit cardio protective and therapeutic effects for chronic heart failure (Kitakaze and Hori, 2000). Besides that, this fungus has been reported can lower serum and plasma glucose in diabetic and epinephrine-induced hyperglycemic mice (Kiho *et al.*, 1993). The popularity of *C. sinensis* is manifested by the emergence of many its based commercial products in the market including Esulin. The Esulin also contains small amount of other herbs like *Fractus momordica charantia*, *Radix glehnia littoralis*, *Rhizoma dioscorea opposita*, *Radix ophiopogon japonicus* and *Radix rehmannia preparata*.

These compound formulations are generally used for treatment of diabetes and this combination is preferable to provide synergistic therapeutic effects. Several previous studies related to *C.sinensis* concentrated only on the ability of this herb in different pharmacological aspects. However, the basic information on the types of phytochemical groups and mineral elements present in this herb is scanty. Thus, the aims of this study were to investigate the presence of phytochemical constituents and mineral contents of *C.sinensis* and its based product, Esulin.

MATERIALS AND METHODS

Materials

The raw materials of *C. Sinensis* and Esulin in the form of dried powder were supplied by AIFA Health Sdn. Bhd, Cheras, Selangor, Malaysia. The powder is yellowish green and was stored in an air tight container at room temperature before extraction process.

Preparation of aqueous extract

The preparation of aqueous extract of these two products, *C.sinensis* and Esulin were prepared according to Sripanidkulchai *et al.* (2000), with slight modifications. About 100 g of each product was boil in 1L of distilled water (DW) approximately three to four hours until the volume was 50% reduced. After leaving it to cool down to room temperature, the decoction was filtered through filter paper and then centrifuged at 2800 rpm for five minutes. The supernatant was subsequently rotavaporised until a percentage yield of extracts ranged from 7-19% w/w was obtained.

Phytochemicals screening

Screening of phytochemicals for both extracts was carried out using standard procedures as described below. The extracts used were diluted with distilled water with a ratio 1:100 (w/v) respectively prior to the screening process.

Test for alkaloids

To the 2 ml of each extract, 1.5 ml of 1 % hydrochloric acid was added. After heating the solution in water bath, 6 drops of Mayer's reagent was added. Formation of orange colouration indicated the presence of alkaloids (Rasool *et al.*, 2010).

Test for saponins

Approximately 0.2 ml of each extract was boiled in 5 ml of distilled water and filtered. It was shaken vigorously for 5 min. Persistence of foams was an indicator for saponins (Sofowora, 1993).

Test for tannins

About 0.5 g of each extract was boiled in 20 ml of distilled water and filtered. A drop of 0.1% ferric chloride was added and observed a greenish black or blue black colouration signified the presence of tannins (Trease and Evans, 1989).

Test for terpenoids (Salkowski test)

Five (5) ml of each extract was mixed in 2 ml of chloroform. Then 3 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration between upper and lower layer was formed to show the presence of terpenoids (Harborne, 1973).

Test for flavonoids

Five (5) ml of each extract was added to 5 ml of diluted ammonia solution followed by addition of concentrated sulphuric acid. The mixture was gently shaken and a yellow colouration indicated the presence of flavonoids (Sofowora, 1993).

Elemental analysis

The methodology for elemental analysis was modified from Shahidi *et al.* (1999). Approximately 0.5 g of each extract was subjected to dry ash in well cleaned porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5ml nitric acid/hydrogen peroxide (HNO₃/ H₂O₂) (1:1) and heated gently on hot plate until brown fumes disappeared. Five (5) ml of distilled water was added and heated until colourless solution was obtained. The digested samples were then diluted to 100 ml distilled water and filtered through a Whatman filter paper. This solution was used for elemental analysis by atomic absorption spectrophotometer (AAS).

RESULTS

The phytochemical screening revealed the presence of medicinal active constituents in both extracts of *C.sinensis* and Esulin. Table 1 exhibited the groups of phytochemicals that were identified in the herb extracts. Alkaloids, saponins, tannins and flavonoids were detected in both extracts. Whereas, terpenoids only present in aqueous extract of Esulin.

Table 1: Qualitative analysis of the phytochemicals of *Cordyceps sinensis* and Esulin extracts

Constituent	<i>C.sinensis</i> aqueous extract	Esulin aqueous extract
Alkaloids	+	+
Saponins	+	+
Tannins	+	+
Terpenoids	-	+
Flavonoids	+	+

+ = Present; - = Absent

The elemental results obtained are presented in Table 2. Six different types of heavy metals were found in all tested samples and their amount were below the maximum permissible level fixed by WHO (1989) and Malaysian Food Law (1985). In both extracts tested, ferum (Fe) showed the highest concentration while cadmium (Cd) level was the lowest. The decreased in heavy metals of the extracts were in order, ferum (Fe)>lead (Pb)> nickel (Ni)>zinc (Zn)>copper (Cu)>cadmium (Cd).

Table 2: Elemental analysis of *C.sinensis* and Esulin

Plants	ppm (mean+SD of 3 replicate)					
	Cu	Zn	Cd	Fe	Pb	Ni
<i>C.sinensis</i>	0.097±0.002	0.339±0.001	0.041±0.005	2.541±0.002	1.648± 0.053	0.527±0.009
Esulin	0.217 ± 0.001	0.277±0.002	0.027±0.007	1.416±0.001	1.606±0.035	0.488±0.002

Cu= copper, Zn= zinc, Cd= cadmium, Fe= ferum, Pb= lead, Ni= nickel

DISCUSSION

The presence of assorted phytochemical components are known to exhibit pharmacological and physiological activities (Sofowora, 1993). Aqueous extracts of *C.sinensis* and Esulin were found to contain flavonoids that exhibit biological activities such as hepatoprotective, anti-inflammatory, antibacterial, antiviral and antidiabetic activities (Yihang *et al.*, 2006). Both extracts possess a potent antioxidant activity and they can protect the cell from the damage due to oxidation process in the body triggered by free radicals (Yu *et al.*, 2000). Furthermore, alkaloids can be found mostly in fungi and plants and it is useful for the treatment of various ailments and disease conditions. Alkaloids also known as a toxic substance but however it often has a pharmacological effect and is used in medicine. The extracts were also revealed to contain tannins, it is commonly referred as tannic acid and water soluble polyphenols that are present in many plant foods. As being reported in experimental animals, tannins responsible to decrease food intake, growth rate, net metabolism energy and protein digestibility of rats and mice (Chung *et al.*, 1998). In addition, terpenoids was absent in *C.sinensis* aqueous extract, even though terpenoids are usually the largest group of natural product and it has anti-inflammatory, antioxidant activities and inhibition of cholesterol synthesis (Haughton *et al.*, 2003). Apart from the phytochemical components, the concentration of trace elements in the plants is as important as well. The elements such as Zn and Fe are essential in enzyme metabolism.

As in this study, Zn content from examined samples was ranged from 0.2 to 0.4 ppm and this amount is considered acceptable. Zn is an essential element needed by human body and is commonly found in nutritional supplements. Zn plays an important role in nucleic acid metabolism. It is also a membrane stabilizer and a stimulator of the immune response (Das and Dasgupta, 2002). However, taking too much Zn into human body through food, water and supplements can affect health. Acute Zn poisoning includes stomach cramps, nausea, diarrhea, fever, vomiting and lethargy (Obi *et al.*, 2006). Ingesting high levels of Zn for several months may cause anemia, decrease levels of high-density lipoprotein (HDL) cholesterol and damage the pancreas (Roney *et al.*, 2005).

As of Zn, Fe also plays a number of essential roles in human body, including oxygen transport and cell growth. Sixty six percent of Fe stores remain in haemoglobin and protein in red blood cells that carries oxygen to tissues and organs. In this study, the Fe content found in tested samples was comparatively low as compared to the maximum permissible level (MPL) of iron which is 7.0 ppm (WHO, 1989). This detected amount 1.0 to 3.0 ppm is regarded safe for human consumption. However, too much Fe can cause serious harm, even death. Fe has astringent action resulting in irritation of the gastrointestinal mucosa; can give rise to gastric discomfort, nausea, vomiting and diarrhea or constipation (Annan *et al.*, 2010).

Although Cu is an essential enzymatic element for normal plant growth and development but it is toxic at elevated concentration. In this study, the highest concentration of Cu was found in aqueous extract of Esulin which is 0.217 ppm, however it was still at safe and acceptable level. Cu has since been found to be a constituent of many important enzymes including cytochrome c oxidase, superoxide dismutase (cytoplasm), ceruloplasmin, dopamine B-hydroxylase, lysyl oxidase, tyrosine and monoamine oxidase. Gastrointestinal distress is one of the most commonly adverse health effects reported by copper. Other Cu poisoning includes irritation to respiratory tract, nausea, vomiting, abdominal pain, coughing, sneezing, runny nose, pulmonary fibrosis and increased vascularity of nasal mucosa (Roney *et al.*, 2005).

Cd is also found in all tested samples of this study. As presented in Table 2, the content of Cd in both samples is from 0.02 to 0.05 ppm. WHO, (1989) prescribed limit for Cd content in medicinal plant is 0.3 ppm. Thus, value of Cd in the samples is acceptable. Cd is a non essential trace element with uncertain direct function in both plants and humans (Annan *et al.*, 2010). Most of Cd that enters human body goes to the kidney and liver and can remain there for many years. A small portion of the Cd that enters human body leaves slowly in urine and feces. However, excessive Cd can overload the ability of liver and kidney to change it to a harmless form and therefore may affect human health. High Cd levels severely irritate the stomach, leading to vomit and diarrhea, and sometimes cause death (Taylor *et al.*, 1999).

According to WHO, (1992) the permissible limit of Pb for medicinal plants, based on the ADI (Acceptable Daily Intake) is 10 ppm. The value obtained for Pb from the herbal samples in this study is from 1.5 to 1.7 ppm and therefore it is regarded safe for human consumption. Pb is a non essential trace element with uncertain direct function in human body. Too much Pb in human body can cause toxicity and the main target for its toxicity is the nervous system. Pb exposure may cause weakness in fingers, wrists, or ankles. At high levels of exposure, Pb can severely damage the brain and kidneys in adults or children and ultimately cause death, in pregnant women may cause miscarriage and for men can damage the organs responsible for sperm production (Abadin *et al.*, 2007).

The Ni content found in all tested samples was relatively in good range which is between 0.4 to 0.6 ppm. The maximum permissible level (MPL) of nickel is 1.0 ppm (WHO, 1989). The Environmental Protection Agency (EPA) has recommended daily intake of Ni should be less than 1.0 ppm which beyond this limit may cause toxicity (McGrath and Smith, 1990). Ni mostly presents in the pancreas and hence, plays an important role in the production of insulin. It also may promote prolactin production thus involved in human breast milk production (Haas and Levin, 2006). Ni is an essential trace element in animals although the functional importance of nickel has not been clearly demonstrated. The presence of Ni in *C.sinensis* and Esulin suggests that these herbal products could be effective in ameliorating diabetic complication.

CONCLUSION

The present study has shown the presence of five types of essential phytochemicals and six elemental compositions in the aqueous extracts of *C. sinensis* and its based product, Esulin. The results obtained from both extracts may provide justifications for their pharmaceutical responses. Further research is needed to investigate and explore the abilities and benefits of *C.sinensis* and Esulin as therapeutic agents for pharmaceutical industries.

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REFERENCES

- Abadin, H, Ashizawa, A, Stevens, Y.W, Lladós, F, Diamond, G, Sage, G, Citra, M, Quinones, A, Bosch, S.J. and Swarts, S.G. (2007). Toxicological Profile of Lead. US Department of Health and Human Service, Public Health Service Agency for Toxic Substances and Disease Registry 1: 1-528
- Annan, K, Kojo, A.I, Cindy, A, Samuel, A. and Tunkumgnen, B.M. (2010). Profile heavy metals in some medicinal plants from Ghana commonly used as components of herbal formulations. *Pharmacognosy* 2(1): 41-44.
- Bhandari, A. K, Negi, J.S, Bisht, V. K, Rana, C.S, Bharti, M.K. and Singh, N. (2010). Chemical constituent, inorganic element and properties of *Cordyceps sinensis*. *Nature and Science* 8(9): 253-256.
- Chung, K.T, Wong, T.Y, Wei, C.I, Huang, Y.W. and Lin, Y. (1998). Tannins and human health: a review. *Food Science Nutrition* 38(6): 421-464.

- Dai, G, Bao, T, Xu, C, Cooper, R. and Zhu, J.S. (2001). Cordyceps Cs-4 improves steady-state bio-energy status in mouse liver. *Journal of Alternative Complementary Medicine* 7(3): 231-240.
- Das, K.K. and Dasgupta, S. (2002). Effect of nickel sulphate on testicular steroidogenesis in rats during protein restriction. *Environmental Health Perspectives* 110(9): 923-926.
- Haas, E.M. and Levin, B. (2006). *Staying Healthy with nutrition: The complete guide to diet and nutritional medicine*. 21st Ed. Celestial Arts, New York.
- Halpern, G. M. (2007). *Healing mushroom, effective treatments for today's illness*. Square One Publishers, Garden City, New York.
- Harborne, J. B. (1973). *Phytochemical Methods: A guide to modern techniques of plant analysis*. Chapman and Hall, New York. ISBN: 0412105403, pp: 279.
- Hill, A. F. (1952). *Economic Botany. A textbook of useful plants and plant product*. 2nd Ed. McGraw-Hill Book Company Inc, New York.
- Houghton, P. J, Mensah, A. Y, Iessa, N. and Hong, L. Y. (2003). Terpenoids in Buddleja: relevance to chemosystematics, chemical ecology and biological activity. *Phytochemistry* 64(2): 385-393.
- Kiho, T, Ookubo, K. and Ukai, S. (1993). Polysaccharides in fungi. Hypoglycaemic activity and chemical properties of a polysaccharides from the cultured mycelium of *Cordyceps sinensis*. *Biological Pharmaceutical Bulletin* 16(12): 1291-1293.
- Kitakaze, M. and Hori, M. (2000). Adenosine therapy a new approach to chronic heart failure. *Expert Opinion on Investigational Drugs* 9 (11): 2549-2599.
- Kobayasi, Y. 1982. Keys to the taxa of genera Cordyceps and Torrubiella. *Mycological Society* 23: 329-364.
- Li, L. S, Zheng, F. and Liu, Z. H. (1996). Experimental study on effect of *Cordyceps sinensis* in ameliorating amino-glycoside induced nephrotoxicity. *Chung Kuo Chung Hsi I Chieh Ho Tsa Chih* 16(12): 733-737.
- McGrath, S. P. and Smith, S. (1990). Chromium and nickel in heavy metals in soils. B.J. Alloway, Blackie, Glasgow. pp: 125
- Malaysian Food Law. (1985). *Food Act and Regulation*. MDC Publisher Printer Sdn Bhd, Kuala Lumpur.
- Obi, E, Akunyili, D. N, Ekpo, B. and Orisakwe, O. E. (2006). Heavy metals hazards of Nigerian herbal remedies. *Science of the Total Environment* 369(1): 35-41.
- Rasool, R, Ganai, B. A, Akbar, S, Kamili, A. N. and Masood, A. (2010). Phytochemical screening of *Prunella vulgaris* an important plant of Kashmir. *Journal of Pharmacology Sciences* 23(4): 399-402.
- Roney, N, Smith, C. V, William, M, Osier, M. and Paikoff, S. J. (2005). *Toxicological profile of zinc*. Agency for Toxic Substance and Disease Registry, New York.
- Shahidi, F, Chavan, U. D, Bal, A. K. and McKenzie, D.B. (1999). Chemical composition of beach pea (*Lathyrus maritimus*) plants parts. *Food Chemistry* 64(1): 39-44.
- Sofowora, A. (1993). *Medicinal plants and traditional medicine in Africa*. 3rd Ed. Spectrum Books Ltd, Nigeria. pp:150-153
- Sripanidkulchai, B, Na-nakorn, S, Wongpanich, V. and Tanyakupta, P. (2000). A behavioural investigation on utilization of medicinal plants for dysuria at Pon District, Khon Kaen. *Khon Kaen University Research Journal* 5(1): 4-10.
- Taylor, J, DeWoskin, R. and Ennever, F.K. (1999). *Toxicological profile of Cadmium*. Agency for Toxic Substance and Disease Registry, New York.
- Trease, G. E. and Evans, W.C. (1989). *A Textbook of Pharmacognosy*. 13th Ed. Bailliere Tindall Ltd, London.
- Wadood, A, Ghufuran, M, Jamal, S. B, Naeem, M, Khan, A. and Ghaffar, R. (2013). Phytochemical analysis of medicinal plants occurring in local area of Mardan. *Biochemistry Analytical Biochemistry* 2(4): 1-4.
- WHO(1989). *Evaluation of certain food additives and contaminants*. World Health Organization, Switzerland.
- WHO(1992). *Expert committee and specification for pharmaceuticals preparation*. World Health Organization Technical Report Series 823: 1-134.
- Yihang, W, Fang, W, Qunxiong, Z, Longxi, L, Hongtian, Y, Changxin, Z, Xiemei, W. and Yu, Z. (2006). Hepatoprotective effect of total flavonoids from *Langgera alata* against carbon tetrachloride induced injury in primary cultured neonatal rat hepatocytes and in rats with hepatic damage. *Journal of Biomedical Science* 13(4): 569-578.
- Yu, Y, Satomi, K, Kazuki, N, Kazumasa, S. and Masaru, K. (2000). Antioxidant activity of the extract from fruiting bodies of cultured *Cordyceps sinensis*. *Phytotherapy Research* 14: 647-649.
- Zhu, J, Halpern, G. M. and Jones, K. (1998). The scientific rediscovery of an Ancient Chinese herbal medicine: *Cordyceps sinensis*. *Journal Alternative Complementary Medicine* 4(3): 289-303.

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