

Review on Pharmacologically Active Metabolites from Yarsagumba (*Ophiocordyceps sinensis*), an Epitome of Himalayan Elixir

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Abstract

Yarsagumba (*Ophiocordyceps sinensis* (Berk.) Sung *et al.*) is a well-known entomogenous fungus native to alpine nival terrain of trans-Himalaya and Tibetan Plateau. The traditional use of *O. sinensis* in Ayurvedic medicine as well as in traditional Chinese medicine for range of human health requirements with well documented evidences are themselves testimony of its value. It is regarded as winter worm, summer grass due to its association with *Thitarodes* (*Hepialus*) larvae. The extremities of habitat condition and endurance of fungus to sustain adversaries by production of metabolites has led to unique profile of metabolites including nucleosides, proteins and nitrogenous compounds, polysaccharides, sterols, fatty acids and their derivatives, as well as some vitamins and inorganics. There are wide ranges of biological activities that have been reported from *O. sinensis* including anti-inflammatory, antioxidant, anti-tumor, anti-metastatic, immunomodulatory, antimicrobial, insecticidal, hypolipidaemic, hypoglycemic, anti-ageing, lipolytic, neuroprotective, renoprotective effects, etc. Although several components can be responsible for activity of compounds, understanding ultimate compounds which fit with biomolecular target is crucial to combat diseases and development of new class of effective drugs. In this review a concise evaluation of pharmacological activities of metabolites reported for *O. sinensis* are done to provide insight into its biologically active components.

Key words: active metabolite, bioactivity, *Cordyceps sinensis*, pharmacology

Introduction

Ophiocordyceps sinensis (Berk.) Sung *et al.* (Ophiocordicipitaceae, Hypocreales, Ascomycota, Kingdom Fungi), the most valued among fungi, is distributed in trans-Himalayan terrain of Nepal, Tibetan Plateau, India and Bhutan in the altitudinal range of

3,000 to 5,000 asl. On the basis of phylogenetic study (Sung *et al.* 2007), *Ophiocordyceps sinensis* was named for *Cordyceps sinensis* (Berk.) Sacc. It is popularly known as Yarsagumba in Nepal. It is also referred as Jivan Buti, Kira Chhyau, Kira Jhar, Saram

Buti, Jingani etc in Nepal (Shrestha *et al.* 2010). It is regarded as highly effective for several human maladies including cancer, hypoglycemia, asthma, hypercholesterolaemia, sexual dysfunction, immunodeficiency, etc (Zhu *et al.* 1998a,b). Further, Holliday and Cleaver (2008) reported several pharmacological activities including improvement of physical performance, circulatory functions, hepatoprotection, renoprotection, atherosclerosis, anti-tumor and anti-metastatic. Another important aspect is its use as tonic, aphrodisiac, cardiogenic and expectorant (Baral & Kurmi 2006) and its market value can even reach US\$ 800 for an ounce (Giove 2011). Traditionally in Nepal, powdered *O. sinensis* is taken along with honey, milk, water or its extract in an alcoholic drink (Devkota 2006). There is also report of administration of its infusion with powdered root of *Dactylorhiza hatagirea* (Paanch aunle) or *Ephedra gerardiana* (Somlata) (Gewali 2008). The scientific name *O. sinensis* refers to the sexual stage including stalked fruiting-body whereas the asexual mycelium culture is termed as *Hirsutella sinensis* (Shrestha *et al.* 2010). The fungal invasion in host larva and its growth occur in late summer, and its growth eventually leads to replacement of internal organs leading to formation of thickened fungal tissue known as endosclerotium during winter (Chen *et al.* 2004); whereas fruiting-body (stroma) sprouts from the prethorax region of infected larvae and matures in late summer (June-August) (Li & Yang 2009). There are about 60 taxa as potential hosts of *O. sinensis* mostly belonging to genus *Thitarodes* (*Hepialus*) (Wang & Yao 2011). They are commonly known as bat-moths, swift-moths or ghost-moths which depend on soil humus and tender roots of *Ranunculus brotherusii*, *Cyananthus macrocalyx*, *Juncus leucanthus* and *Veronica ciliate* etc (Lei *et al.* 2011). The entire fungus-larva combination is collected for medicinal use. The colour of *O. sinensis* varies from light brown to brown or black depending on habitat condition. The distribution limit, and ever increasing demands have prompted the isolation of mycelium strains and large-scale culture. As cost involved in getting wild specimens is tremendous, the majority of researches on *O. sinensis* are based on mycelium culture and some similarity in effectiveness and content have been reported for both wild and cultured *O. sinensis* (Li *et al.* 2002). However, the charm of natural *O. sinensis* remains intact and there is a billion dollar trade in practice. The question what is present in *O. sinensis*

which makes it so popular or effective has still not been answered exactly although there have been several researches to find out the cause. Several biological activities are based on extract, fractions and polysaccharides and there are discrepancies in structure determination of polysaccharides (Chen *et al.* 2013). The fruit-body production in cultivation has not been successful yet and mycelia culture is the major source of studies (Dong & Yao 2011). In comparison to cultured mycelium, the natural *O. sinensis* undergoes cascade of events from the selection of host up to the formation of fruiting enduring extreme environmental conditions together with interaction with *Cordyceps*-associated species. There is great likelihood of presence of active small molecule compounds in wild *O. sinensis* but isolation and identification of such compounds are still awaited due to enormous cost of wild harvest. There is also equal chance of synergetic effect of several compounds responsible for some of the activities. The recent report of the major pharmacological activities of *O. sinensis* and a comprehensive summary compounds responsible are reviewed here which are presented as shaded matrix to elucidate possible active components.

The major metabolites include proteins and nitrogenous compounds, polysaccharides, sterols, nucleosides, fatty acids and their derivatives, vitamins and inorganics (Zhu *et al.* 1998a,b, Holliday & Cleaver 2008). The nucleosides are regarded as the active components and adenosine has been used as chemical marker for quality control of *O. sinensis* (Shiao *et al.* 1994). In addition to adenosine, Li *et al.* (2006a) reported other marker compounds of wild *O. sinensis* as ergosterol, adenosine, cordycepin, guanosine, inosine, uridine, mannitol and polysaccharides which have been reported for their biological activities (Zhao *et al.* 2013). The relatively higher amount of inosine (0.33 mg/g), ergosterol (3.65-10.34 mg/g), mannitol (38.64-35.42 mg/g) in natural *O. sinensis* in comparison to cultured ones producing 0.03-0.19 mg/g of inosine, 0.38-1.31 mg/g of ergosterol, 10.24-13.41 mg/g of mannitol are noteworthy (Li *et al.* 2006a). One of the active compounds, cordycepin (**13**), which is a major component of *Cordyceps militaris* (Wang *et al.* 2009a), was reported in small quantity (0.04-0.06 mg/g) in wild but absent in cultured *O. sinensis* (Li *et al.* 2006a). However, Kim & Yun (2005) reported 18.19 mg/l cordycepin in cultured *O. sinensis* and mentioned

variation in cordycepin content depending on strain. Some other noteworthy constituents in *O. sinensis* are carbohydrates, D-mannitol, trehalose, uridine, adenine, adenosine and guanosine (Wang *et al.* 2009a). Chen *et al.* (2009) reported two new epipolythio dioxopiperazines, gliocladicillins A, B and 11,112-dideoxyverticillin from *Cordyceps*-associated fungi which indicated possibly beneficial *O. sinensis* associated species. Yang *et al.* (2011) isolated 50 compounds, including five new constituents, cordysinins A-E. Yu *et al.* (2012) reported fifty-one volatile compounds including aldehydes, alcohols, ketones, esters, aromatics, phenols, acids, alkanes, alkenes, furans and other phenolic compounds from the mycelia cultured by solid-state media and submerged fermentation. Although there are several possibilities of synergetic relation of compounds working to give some activities, the lock-and-key theory and one hit target to combat certain disease is required in practice for effective use of well defined drugs, their production and commercialization for the benefit of mass population.

Dose and toxicity

The acute toxicity in bone marrow chromosome aberrations and Ames test showed *O. sinensis* as safe (Shen *et al.* 2001). Lie *et al.* (2005) reported non toxic nature of polysaccharide from *O. sinensis* in test for animal mass, biochemical properties of blood, and histopathological changes of liver and kidney. Further, for median lethal dose (LD₅₀), mice injected up to 80 g/kg per day, did not show any fatalities after 7 days of treatment (Holliday *et al.* 2005). Meena *et al.* (2013) also highlighted on non toxic nature of laboratory cultured mycelia of *O. sinensis* oral administration up to 2 g/kg body weight in adult female Wister rats. The human equivalent dose (HED) of 20 mg/kg in mice is 1.62 mg/kg calculated on the basis of body surface area, or it is equivalent to 113 mg for 70 kg person (Liu *et al.* 2006). There are very few reports of toxic side effects like dry mouth, nausea or diarrhea (Zhu *et al.* 1988 a,b). Rabbit given 10 g/kg per day (n=6) did not show any abnormalities in blood test or in kidney or liver function but reported a case of systemic allergic reaction after taking a strain of Cs-4 (Huang *et al.* 1987). However,

no published data are available on effect on children as well as pregnant or lactating women. Intake of 6 g per day of *O. sinensis* has been reported for therapeutic purpose in treatment of cancer (Holliday & Cleaver 2008).

The quality control and authentication of *O. sinensis* product is one of the major issues for safety and pharmacological efficacy. The uses of multiple chemical markers such as nucleosides, ergosterol, mannitol and polysaccharides used for quality control also have some drawbacks (Li *et al.* 2006a). Fu *et al.* (2011) reported process for more precise authentication of sample continuous monitoring of cellular impedance in real time, which produces specific time/dose dependent cell response profiles (TCRPs) in addition to spectroscopic fingerprinting of active compounds by HPLC using adenosine (or 3-deoxyadenosine) as a standard, in accordance with Pharmacopoeia of the People's Republic of China (2005 version). The use of bar-coding and fingerprinting to some extent are in practice now-a-days with accession of ITS and 18s ribosomal RNA gene (MMDBD 2012).

Pharmacologically active metabolites

The chemical constituents profile shows variation in natural and cultured *O. sinensis*. Guan *et al.* (2010) reported occurrence of more than 7.99% free mannitol in natural but less than 5.83% mannitol in cultured *O. sinensis*. Zhu *et al.* (1998a) classified seven classes of chemical constituents in natural and mycelium fermentation of *O. sinensis* as sterols, nucleosides, sugar derivatives, fatty acids, organic acids, proteins and vitamins. Shiao *et al.* (1994) reported similarity in nucleoside patterns in fruiting body and cultured mycelium and also reported presence of significant amount of adenosine (2.47 mg/g) in fruiting bodies. There can be variation in constituents of *O. sinensis* depending on the place of origin and type of strain used for mycelium culture. In traditional Chinese medicine practice, the therapeutic effects reported for *O. sinensis* are due to contribution of multiple components. There exist extensive reviews on clinical aspects of *O. sinensis* (Zhu *et al.* 1998a,b, Holliday 2008, Li & Yang 2009, Wang *et al.* 2012b) as well as on activity of extracts (Lin & Li 2011). In previous review (Shrestha *et al.* 2012) we provided overview of compounds reported for *O. sinensis*. As there are limited reviews specifically dealing with pharmacologically active metabolites, in this brief review we have tried to summarize major effective components that have been reported for *O. sinensis* (Matrix 1).

Matrix 1. Shaded matrix display of major pharmacological activities of compounds from *Ophiocordyceps sinensis**

Compounds	Pharmacological Activities											
	Anti-cancer ^A	Aphrodisiac ^B	Anti-inflammation ^C	Antioxidant ^D	Hepatoprotection ^E	Hypoglycemic ^F	Anti-asthma ^G	Hypolipidemic ^H	Immunomodulatory ^I	Hypocholesterolem ^J	Renoprotection ^K	Anti-hypertension ^L
ergosterol (1)												
ergosterol peroxide (2)												
5 α ,8 α -epidioxy-22- <i>E</i> -ergosta-6,9(11), 22-trien-3 β -ol (3)												
ergosteryl-3- <i>O</i> - β -D-glucopyranoside (4)												
5 α ,8 α -epidioxy-24(<i>R</i>)-methylcholesta-6,22-dien-3 β -D-glucopyranoside (5)												
(3 β ,5 α ,6 β ,22 <i>E</i>)-ergosta-7,22diene-3,5,6 triol (cervisterol) (6)												
ergosta-4,6,8(14),22-tetraen-3-one (7)												
ergosta-5-8(14),22-trien-7-one, 3 β -ol (H1-A) (8)												
β -sitosterol (9)												
stigmasterol (10)												
campesterol (11)												
adenosine (12)												
cordycepin (13)												
cordyceamides A (14)												
cordyceamides B (15)												
cordycedipeptide A (16)												
1-(5-hydroxymethyl-2-furyl)- β -carboline (perlolyrine) (17)												
cordymin (18)												
3',4',7-trihydroxyisoflavone (19)												
11,11'-dideoxyverticillin (20)												
CS-F10 (Gal:Glc:Man = 43:33:24) (21)												
CS-F30 (Gal:Glc:Man = 62:28:10) (22)												
CSP-1 (Glc:Man:Gal = 1:0.6:0.75) (23)												
cordysinocan (Glc:Man:Gal = 2.4:2.1) (24)												
CPS1 (Glc:Man:Gal = 2.8:2.9:1) (25)												
PS-A (Glc:Gal:Man = 2:1:1) (26)												
CS-PS (Man:Rhm:Ara:Xyl:Glc:Gal = 38.37:2.51:2.21:5.22:27.44:24.45) (27)												
D-mannitol (28)												
butylated hydroxytoulaine (29)												

*Each compound (numbered in bold inside parentheses) is followed by reference and pharmacological activities^{A-L}: (1) Zhao *et al.* (2009)^K, (2) Kobori *et al.* (2007)^{A,C}, (3) Matsuda *et al.* (2009)^A, (4) Yang *et al.* (2011)^{C,D}, (5) Matsuda *et al.* (2009)^A, Bok *et al.* (1999)^A, (6) Lin *et al.* (2004)^G, Lin (2002)^K, (7) Zhao *et al.* (2011)^A, (8) Lin *et al.* (1999)^E, Yang *et al.* (2003)^I, (9) Awad *et al.* (2009)^A, Zhang *et al.* (2011a)^B, Fraile *et al.* (2012)^I, (10) Gabay *et al.* (2010)^C, Ostlund (2007)^J, (11) Ostlund (2007)^J, (12) Maggirwar *et al.* (1994)^D, Yue *et al.* (2008)^G, (13) Matsuda *et al.* (2009)^A, Holliday & Cleaver (2008)^A, Leu *et al.* (2011)^B, Kim *et al.* (2006)^C, Yang *et al.* (2011)^C, Xiao *et al.* (2012)^D, Yun *et al.*, (2003)^F, Yue *et al.* (2008)^G, Zhou *et al.* (2008)^I, Kim *et al.* (2009)^L, (14) Jia *et al.* (2005, 2009)^A, (15) Jia *et al.* (2005, 2009)^A, (16) Jia *et al.* (2005)^A, (17) Yang *et al.* (2011)^{C,D}, (18) Wang *et al.* (2012a)^{C,D}, (19) Yang *et al.* (2011)^D, (20) Chen *et al.* (2009)^A, (21) Kiho *et al.* (1999)^{E,F}, (22) Kiho *et al.* (1996)^{E,I}, (23) Li *et al.* (2006b)^{D,F}, (24) Cheung *et al.* (2009)^{A,D,I}, (25) Wang *et al.* (2009b)^D, (26) Kim (2010)^{D,I}, (27) Zhang *et al.* (2011b)^{D,I}, (28) Ohkuma *et al.* (1998)^B, Ilowite *et al.* (2008)^G, Lameire *et al.* (2003)^K, Rangel-Castilla *et al.* (2008)^I, (29) Babu & Wu (2008)^D, Jilal & Devraj (1996)^{H,I}; ↓ immunosuppression, ↑ immunostimulation.

Discussion

Several pharmacological effects have been described for active fractions of *O. sinensis* which need further research for finding exact responsible metabolites. Shashidhar *et al.* (2013) emphasized on need of chemical characterization and mechanisms behind therapeutic effects of active components at molecular level. We have considered here only activities reported for isolated compounds and those with well defined composition. The effective nature of *O. sinensis* can be attributed to several metabolites for anti-cancer, aphrodisiac, anti-inflammation, antioxidant, hepatoprotection, anti-hypoglycemic, anti-asthemic, immunomodulatory, hypolipidemic, hypocholesterolemic, renoprotective and anti-hypertension (Matrix 1). Among pharmacological effects, immunomodulating effect is of special mention as it can control autoimmune disorders as well as inflammation. The reports of role of *O. sinensis* in cure of autoimmune disorder by ergosta-5-8(14),22-trien-7-one, 3 α -ol (**8**) by modulation of the signal transduction proteins Bcl-2 and Bcl-XL (Yang *et al.* 2003) and immunosuppressive function of cordycepin (**13**) by increased interleukin (IL)-10 expression, decreased IL-2 expression and suppression of T lymphocyte activity leading to prevention of graft rejection in organ transplant are noteworthy (Zhou *et al.* 2008). There is also presence of immunostimulant compounds such as cordysinocan (**24**) (Cheung *et al.* 2009) and CS-PS (**27**) (Zhang *et al.* 2011b) in *O. sinensis*. The differential regulation of dendritic cells depending on physiological condition as identified by Li *et al.* (2009) or variation in metabolites produced by different strains can also be the reason of immunostimulating or immunosuppressing effects. As inflammation is also one of the hallmark of aging (Zhang *et al.* 2013a) and presence of compounds like ergosterol peroxide (**2**), ergosteryl-3-*O*- β -D-glucopyranoside (**4**), stigmasterol (**10**), cordycepin (**13**), perlolyrine (**17**), and cordymin (**18**) with the anti-inflammatory property can have role in anti-ageing effect of *O. sinensis*. Cordycepin is one of the most active compound with several activities (Yun *et al.* 2003, Kim *et al.* 2006, Holliday & Cleaver 2008, Yue *et al.* 2008, Kim *et al.* 2009, Leu *et al.* 2011, Yang *et al.* 2011, Xiao *et al.* 2012). There is possibility of its role in synergetic effect with other compounds for several therapeutic effects. Further, the presence of D-mannitol (Guan *et al.* 2010) and butylated hydroxytoluene (Yu *et al.* 2012) in considerable amount

in *O. sinensis* can be another possible reason for its acclaimed healing properties as well as vigor and vitality. The effectiveness of *O. sinensis* as infusion with other herbs also deserves attention. A mechanism for grading *O. sinensis* from different parts of Nepal and quality certification of standard is needed to give product deserved value. There are reports of isolation of several strains of *Cordyceps* (Singh *et al.* 2009, Meng *et al.* 2013), significant genetic differentiation (Zhang *et al.* 2013b) and variation in their pharmacological effect (Meng *et al.* 2013). Therefore, search for potential strains of *Cordyceps* in Himalayas with beneficial metabolites and adequate growth for commercial production are needed to provide alternative to declining wild *O. sinensis*. There should be national interest in further research for finding active components from wild *O. sinensis*, as there is still limited study of constituents based on wild resource (Zhao *et al.* 2013). As *O. sinensis* is revered as an epitome of Himalayan elixir due to presence of several pharmacologically active compounds with potential anti-cancer, antioxidant, immunomodulatory as well as aphrodisiac properties, the in-depth further research for scrutinizing active metabolites is anticipated.

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