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Chemical Constituents of Yarsagumba (*Ophiocordyceps sinensis* (Berk.) Sung *et al.*), a Valued Traditional Himalayan Medicine

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Abstract

Ophiocordyceps sinensis (Berk.) Sung *et al.* is popularly known as Yarsagumba (winter worm summer grass) in Nepal. It is a well-known entomogenous fungus distributed in alpine nival zone of trans-Himalayas and Tibetan Plateau. Its occurrence in extreme biological niche and cohabitation with insect [*Thitarodes* (*Hepialus*)] larvae has led to unique assemblage of metabolites including proteins and nitrogenous compounds, polysaccharides, sterols, nucleosides, fatty acids and their derivatives, vitamins and inorganics. In traditional Chinese medicine, it is one of the most trusted main ingredients for several preparations of remedy from wide range of human health conditions. Several biological activities from *O. sinensis* have been reported that include anti-inflammatory, antioxidant, anti-tumor, anti-metastatic, immunomodulatory, antimicrobial, insecticidal, hypolipidaemic, hypoglycemic, anti-ageing, lipolytic, neuroprotective, renoprotective effects, etc. The chemical constituents and their pharmacological uses are reviewed here highlighting the potentiality of this highly esteemed traditional Himalayan medicine.

Key words: *Cordyceps sinensis*, *Ophiocordyceps sinensis*, *Thitarodes* larvae, Yarsagumba

Introduction

Ophiocordyceps sinensis (Berk.) Sung *et al.* (Ophiocordicipitaceae, Hypocreales, Ascomycota, Kingdom Fungi) is a well-known entomogenous fungus distributed in alpine nival zone of trans-Himalayan terrain and Tibetan plateau in the altitudinal range of 3,000 to 5,000 asl. It has been commonly known by its synonym *Cordyceps sinensis* (Berk.) Sacc. both in scientific and non-scientific communities but has recently been transferred to new genus *Ophiocordyceps* on the basis of phylogenetic study (Sung *et al.* 2007). It is popularly known as Yarsagumba in Nepal, India and Bhutan, and is one of the most-prized herbs. In Ayurvedic literature, this herb was mentioned as Bhu-Sanjivani (Shrestha *et al.* 2010) and its uses were mentioned as Sannipatik Brikka Mahaphiranga, i.e., it cures severe and incurable kidney and syphilis diseases (Shrestha 2010, 2011). Other common Nepali names are Jivan Buti, Saram Buti, Kira Chhyau, Kira Jhar, Jingani, etc (Shrestha *et al.* 2010). The standard Chinese name is Dong Chong Xia Cao (Chong Cao in short), literally meaning winter-worm summer-grass. Japanese and Korean names for this herb are Tochu-Kaso and Dong Chung Ha Cho respectively (Shrestha *et al.* 2010). The literal meaning of Yarsagumba in Tibetan language is coined on the basis of its appearance as a plant in the summer and an insect in the winter. It is regarded as highly effective herbal medicine for several diseases including cancer, hypoglycemia, asthma, hypercholesterolaemia, sexual

dysfunction, immunodeficiency, etc (Zhu *et al*. 1998a,b). It is well known as tonic, aphrodisiac, cardiotonic and expectorant (Baral & Kurmi 2006) and market value can even reach US$ 800 for an ounce (Giove 2011). In Nepal, traditionally powdered material is taken with honey, milk or water. Alcoholic drink is also known to be prepared by immersing dry *O. sinensis* (Devkota 2006). It is also taken as an infusion with powdered root of *Dactylorhiza hatagirea* (Paanch aunle) or *Ephedra gerardiana* (Somlata) (Gewali 2008).

The scientific name for the sexual stage including stalked fruiting-body is termed as *O. sinensis* whereas its asexual mycelium culture is known as *Hirsutella sinensis* (Wei *et al*. 2011). In late summer, conidia or mycelia of the fungus invade and during winter eventually replace the internal organs of host larvae with thickened fungal tissue known as endosclerotium (Chen *et al.* 2004). When the temperature outside gets warm, fruiting-body (stroma) sprouts from the dorsal surface of prothorax of infected larvae, develops gradually and matures consequently in late summer (June-August) (Li & Yang 2009). Wang & Yao (2011) recognized nearly 60 taxa as potential hosts of *O. sinensis*. The host moths that mostly belong to genus *Thitarodes* (*Hepialus*) are commonly known as bat-moths, swift–moths or ghost–moths. *Thitarodes* larvae feed chiefly on soil humus and tender roots of *Ranunculus brotherusii, Cyananthus macrocalyx, Juncus leucanthus* and *Veronica ciliate* (Wei *et al.* 2011). The entire fungus-larva combination is collected for medicinal use (Fig. 1). Its colour varies from light brown to brown or black which can be due to habitat difference as well as melanin concentration. Recently, Dong and Yao (2012), investigating on the presence of melanin in *O. sinensis*, emphasized that it may have role on strong endurance of *O. sinensis* to environmental stress. The growth of

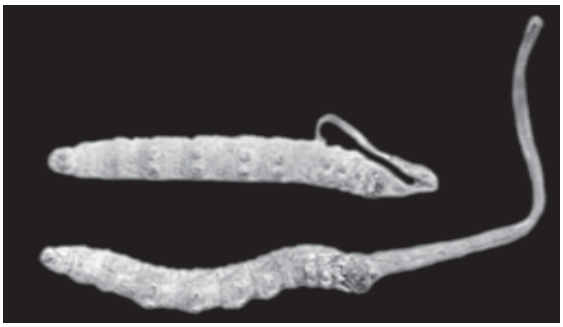


Fig. 1. *Ophiocordycep sinensis* from Manang. Courtesy of Himalayan Flowers, Trees and Animals (Shrestha *et al.* 2011).

O. sinensis has a very restricted habitat, and the yield is decreasing each year due to extensive harvest. Therefore, isolation of mycelium strains is done for large-scale culture and cultivation. The major constituents of *O. sinensis* and their pharmacological effects are dealt here to provide a concise insight into its potentiality as a time-honoured herb.

Phytochemistry

O. sinensis collected from wild is limited and expensive. Hence, cultured mycelium is choice for most of the studies. Phytochemical analysis of *O. sinensis* had led to isolation of proteins and nitrogenous compounds, polysaccharides, sterols, nucleosides, fatty acids and their derivatives, vitamins and inorganics (Zhu *et al.* 1998a,b, Holliday & Cleaver 2008). Nucleosides are regarded as the active components and adenosine has been used as a marker for quality control of *C. sinensis* (Shiao *et al.* 1994). The chemical components such as ergosterol, adenosine, cordycepin, guanosine, inosine, uridine, mannitol and polysaccharides are major components of wild *C. sinensis* (Li *et al.* 2006). The inosine (**33**) content in natural *O. sinensis* contains much higher amount (0.33 mg/g) than culture ones (0.03-0.19) and cordycepin (**27**) content was also present in small quantity (0.04 mg/g) in wild but absent in cultured *O. sinensis* (Li *et al.* 2006). The high-performance liquid chromatography (HPLC) coupled with diode array detection (DAD) and evaporative light scattering detection (ELSD) method for qualitative and quantitative analysis showed carbohydrates, mannitol and trehalose are rich in *O. sinensis* in comparison to *Cordyceps gunnii* and *C. liangshanensis* and cultured *Cordyceps* mycelia. Further, uridine (**34**), adenosine (**26**) and guanosine (**31**) were the major components in cultured *Cordyceps* whereas cordycepin (**27**) was the major component in *C. militaris* (Wang *et al.* 2009). It is noteworthy that in LC/ESI-MS analysis *O. sinensis* had more adenine (**23**) content than *C. militaris* and hypoxanthine (**25**) was present in *O. sinensis* but not detected in *C. militaris* (Huang *et al.* 2003). Yang *et al.* (2011) isolated 50 compounds, including five new constituents, cordysinins A-E (**39-43**). They reported the presence of the significant amount of ergosterol (**1**), ergosteryl-3-*O*-*â*-*D*-glucopyranoside (**2**), *â*-sitosterol (**12**), stigmasterol (**15**), uracil (**22**), *p*-hydroxybenzoic acid (**81**), 3-hydroxy-2-methyl-4-pyrone (**92**), cyclo(L-Pro-L-Val) (**65**), cyclo(L-Pro-L-Tyr) (**67**), and D-mannitol (**112**) of in *n*-hexane/MeOH-H_2O and ethyl acetate fractions. Further, fifty-one volatile compounds including aldehydes, alcohols,

ketones, esters, aromatics, phenols, acids, alkanes, alkenes and the others were identified from the mycelia cultured by solid-state media and submerged fermentation, respectively (Yu *et al.* 2012). Previous research results have reported several pharmacological activities including improvement of physical performance, circulatory functions, atherosclerosis inhibition, respiratory system, kidney and renal system and anti-tumor, anti-metastatic (Holliday & Cleaver 2008). The reported compounds are categorized here as isolated constituents (**1-112**) and detected volatile components (**V1-V51**) in Table 1.

Table 1. Major chemical constituents of *Ophiocordyceps sinensis*

Compounds	Reference
Sterols: ergosterol (**1**), ergosteryl-3-*O*-*â*-D-glucopyranoside (**2**), ergosterol peroxide (**3**), 5*á*,8*á*-epidioxy-24(*R*)-methylcholesta-6,22-dien-3*â*- D-glucopyranoside (**4**), (24*R*)-ergosta-7,22-diene-3*â*,5*á*,6*â*-triol (cerevisterol) (**5**), ergosta-4,6,8(14), 22-tetraen-3-one (**6**), 4,4-dimethyl-5á-ergosta-8,24(28)-dien-3â-ol (**7**), 3-*O*-ferulylcycloartenol (**8**), 5*á*,6*á*-epoxy-24(*R*)-methylcholesta-7,22-dien-3*â*-ol (**9**), ergosta-5-8(14),22-trien-7-one, 3*â*-ol [H1-A] (**10**) 22,23-dihydroergosteryl-3-*O*-*â*-D-glucopyranoside (**11**), *â*-sitosterol (**12**), *â*-sitosterol 3-*O*-acetate (**13**), daucosterol (**14**), stigmasterol (**15**), stigmasterol 3-*O*-acetate (**16**), cholesterol (**17**), campesterol (**18**), dihydrobrassicasterol [D^5-ergosterol] (**19**); fungisterol [D^7-ergosterol] (**20**),(17*R*)-17-methylincisterol (**21**)	Kadota *et al.* 1986 (**17**, **18**, **19**); Bok *et al.* 1999 (**2**, **3**, **4**, **9**, **11**); Lin *et al.* 1999 (**10**); Li *et al.* 2003 (**1**, **3**, **5**, **12**, **17**); Yang *et al.* 2011 (**6-8**, **12-16**, **20-21**)
Nitrogenous compounds: uracil (**22**), adenine (**23**), guanine (**24**), hypoxanthine (**25**), adenosine (**26**), cordycepin (**27**), dideoxyadenosine (**28**), N^6-(2-hydroxyethyl) adenosine (**29**) inosine (**30**), guanosine (**31**), thymine (**32**), thymidine (**33**), uridine (**34**), dideoxyuridine (**35**), cordyceamides A (**36**), cordyceamides B (**37**), aurantiamide acetate (**38**), cordysinin A (**39**), cordysinin B (**40**), cordysinin C (**41**), cordysinin D (**42**), cordysinin E (**43**), cordycedipeptide A (3-acetamino-6-isobutyl-2,5-dioxopiperazine) (**44**), 3-isopropyl-6-isobutyl-2,5-dioxopiperazine (**45**), 3,6-di(4-hydroxy)benzyl-2,5-dioxopiperazine (**46**), caffeine (**47**), N-(2'-hydroxy-tetracosanoyl)-2-amino-1,3,4-trihydroxy-octadec-8*E*-ene (tetracosanamide) (**48**), 2-nicotinic acid (**49**)	Zhu *et al.* 1998a (**22, 31, 33-35**); Huang *et al.* 2003 (**23, 25-27**); Li *et al.* 2003 (**47**,**48**); Jia *et al.* 2005 (**44-46**); Li *et al.* 2006 (**28-31**, **34**); Holliday & Cleaver 2008 (**26-29**); Jia *et al.* 2009 (**36-38**); Liu *et al.* 2010 (**22-26, 30**, **34**); Yang *et al.* 2011 (**32**, **39-43**, **49**)
Proteins: nucleic acids, amino acids, polyamines cyclo-(Gly-Pro) (**50**), cyclo-(Leu-Pro) (**51**), cyclo-(Val-Pro) (**52**), cyclo-(Ala-Leu) (**53**), cyclo-(Ala-Val) (**54**), and cyclo-(Thr-Leu) (**55**), 1,3-diamino propane (**56**), cadaverine (**57**), spermidine (**58**), spermine (**59**), putrescine (**60**), flazin (**61**), perlolyrine (**62**), 1-methylpyrimidine-2,4-dione (**63**), 1-acetyl-â-carboline (**64**), cyclo(L-Pro**3**-L-Val) (**65**), cyclo(L-Phe-L-Pro) (**66**), cyclo(L-Pro-L-Tyr) (**67**), cordymin (**68**), L-tryptophan (**69**)	Zhang *et al.* 1991 (**69**); Holliday & Cleaver 2008 (**50-60**), Yang *et al.* 2011 (**61-67**); Qian 2012 (**68**)
Fatty acids and other organic acids: palmitic acid (**70**), lauric acid (**71**), myristic acid (**72**), pentadecanoic acid (**73**), palmitoleic acid (**74**), linoleic acid (**75**), oleic acid (**76**), stearic acid (**77**), docosanoic acid (**78**), lignoceric acid (**79**), succinic acid (**80**)	Li *et al.* 2003 (**70**); Yang *et al.* 2009 (**70-79**) Yang *et al.* 2011 (**80**)
Phenolics and acids: *p*-hydroxybenzoic acid (**81**), vanillic acid (**82**), syringic acid (**83**), *p*-methoxybenzoic acid (**84**), *p*-hydroxyphenylacetic acid (**85**), 3,4-dihydroxyacetophenone (**86**), 4 hydroxy-acetophenone (**87**), protocatechuic acid (**88**), 3, *p*-methoxyphenol (**89**), acetovanillone (**90**), salicylic acid (**91**), 3-hydroxy-2-methyl-4-pyrone (**92**), methyl *p*-hydroxyphenyl-acetate (**93**), 2-deoxy-D-ribono-1,4-lactone (**94**), furancarboxylic acid (**95**)	Yang *et al.* 2011 (**81-95**)
Isoflavones: 32 ,42 ,7-trihydroxyisoflavone (**96**), glycitein (**97**), daidzein (**98**), orobol (**99**), genistein (**100**)	Yang *et al.* 2011 (**96-100**)

Polysaccharide and sugar derivatives: CS-F30 [Gal:Glc:Man = 62:28:10](**101**), CS-F10 [Gal:Glc:Man = 43:33:24] (**102**), CT-4N [Man:Gal =3:5] (**103**), CS-81002 [Man:Gal:Glc = 10.3:3.6:6.1] (**104**), SCP-I [ᴅ-glucan](**105**), CSP-1 [GlC:Man:Gal = 1:0.6:0.75] (**106**), CPS1 [Glc:Man:Gal = 2.8:2.9:1] (**107**), cordysinocan [Glc:Man:Gal = 2.4:2.1] (**108**), PS-A [Glc:Gal:Man = 2:1:1] (**109**), CS-PS [Man:Rhm:Ara:Xyl:Glc:Gal = 38.37:2.51:2.21:5.22:27.44:24.45] (**110**), mannoglucan [Man:Glc = 1:9] (**111**), D-mannitol (**112**)	Kiho *et al.* 1986 (**103**); Gong *et al.* 1990 (**104**) ; Kiho *et al.* 1993 (**101**); Kiho *et al.* 1999 (**102**); Li *et al.* 2003 (**106**); Wu *et al.* 2006 (**105**); Wu *et al.* 2007 (**111**); Cheung *et al.* 2009 (**108**); Wang *et al.* 2009 (**107**); Kim 2010 (**109**); Zhang *et al.* 2011 (**110**); Yang et al. 2011 (**112**)
Vitamins: B1, B2, B12, E, and K	Zhu *et al.* 1998a
Inorganics: K, Na, Ca, Mg, Fe, Cu, Mn, Zn, Pi, Se, Al, Si, Ni, Sr, Ti, Cr, Ga, V, and Z	Zhu *et al.* 1998a
Volatile compounds: **Aldehydes:** Benzaldehyde (**V1**), benzeneacetaldehyde (**V2**), (E)-2-dodecenal (**V3**), nonanal (**V4**), (E)-2-Nonenal (**V5**), 4-fluoro-3-hydroxy-benzaldehyde (**V6**), decanal (**V7**), (E,E)-2,4-nonadienal (**V8**); **Alcohols:** phenylethyl alcohol (**V9**), 2-(methylthio)-3-pyridinol (**V10**), p-menth-4(8)-en-9-ol (**V11**), 5-methyl-5-hexen-2-ol (**V12**), 2±, 4², 8±-decahydro-2-naphthalenol(**V13**), 2-Butyl-2,7-octadien-1-ol (**V14**), 4,6-di-tert-butyl-m-cresol (**V15**); **Ketones:** 6-ethenyldihydro-2,2, 6-trimethyl-2H-pyran-3(4H)-one (**V16**), trans-3-nonen-2-one (**V17**), 1-oxaspiro(4,5)decan-2-one (**V18**), 4-(2-furanyl)-3-buten-2-one (**V19**), 7-chloro-2,3-dihydro-3-(4-N,N-dimethylaminobenzylidene)-5-phenyl-1H-1,4-benzodiazepin-2-one(**V20**), 1,5-dihydro-1-methyl-2H-pyrrol-2-one (**V21**), (Z)-dihydro-5-(2-octenyl)-2(3H)-furanone (**V22**), 4-butoxy-3-penten-2-one (**V23**); **Esters:** 2-butynedioic acid, di-2-propenyl ester (**V24**), 1,3-cyclohexadiene-1,3-diol, 5,5-dimethyl-diacetate (**V25**), benzoic acid, 2,4-bis[(trimethylsilyl)oxy]-trimethylsilyl ester (**V26**), oxalic acid, isobutyl tetradecyl ester (**V27**); **Aromatics:** azulene (**V28**), 1-methylene-1H-indene (**V29**), 2,6-dimethyl-naphthalene (**V30**), 1,6-dimethyl-naphthalene (**V31**); **Phenols:** 2-methyl-phenol (**V32**), butylated hydroxytoluene (**V33**); **Acids:** phosphonic acid (**V34**), alkanes: decamethyl-cyclopentasiloxane (**V35**), 1-chloro-nonadecane (**V36**), 2,4-diisopropyl-1,1-dimethyl-cyclohexane (**V37**), dodecamethyl-cyclohexasiloxane, (**V38**), 2-methyl-dodecane (**V39**), tetradecamethyl-cycloheptasiloxane (**V40**), 2,6,10,14-Tetramethyl-hexadecane (**V41**), 1,54-Dibromo-tetrapentacontane (**V42**); **Alkenes:** (E)-9-eicosene (**V43**); **Others:** 2-pentyl-furan (**V44**), 2-hexyl-2,4-decadienal, (E,E)-furan(**V45**), methyl 2,3-anhydro-4-azido-4-deoxy-²-L-Ribopyranoside (**V46**), 1,2-benzisothiazole (**V47**), indole (**V48**), 1-(chloromethyl)-3-methoxy-benzene (**V49**), 1,2-benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-1,1-dioxide (**V50**), disulfide, di-tert-dodecyl (**V51**)	Yu *et al.* 2012 **(V1-V51)**

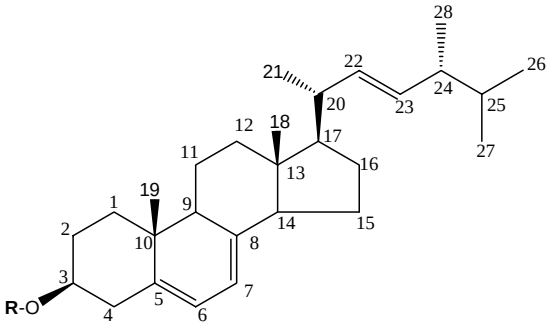
Toxicity

There are very few reports of toxic side effects like dry mouth, nausea or diarrhea (Zhu *et al.* 1988a,b). Rabbit given 10 g/kg per day (n=6) did not show any abnormalities in blood test or in kidney or liver function (Huang *et al.* 1987). Further, for median lethal does (LD_{50}), mice injected up to 80 g/kg per day, did not show any fatalities after 7 days (Holliday *et al.* 2005). However, there is a report of a case of systemic allergic reaction after taking a strain of Cs-4 (Huang *et al.* 1987). Lie *et al.* (2005) observed non toxic nature *O. sinensis* extract in test for animal mass, biochemical properties of blood, and histopathological changes of liver and kidney. Further, test for acute toxicity in bone marrow chromosome aberrations and Ames test also showed it as safe (Shen *et al.* 2001). However, published data on effect on children as well as pregnant or lactating women is still lacking.

The adulteration of wild harvest is one of the major problems in trade of *O. sinensis*. The methodology to ensure authenticity and quality of wild *O. sinensis* and its products for safety and pharmacological efficacy is appearing as outmost importance. However, there are still drawbacks in use of multiple markers such as nucleosides, ergosterol, mannitol and polysaccharides used for quality control (Li *et al.* 2006). 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).

Chemical Constituents

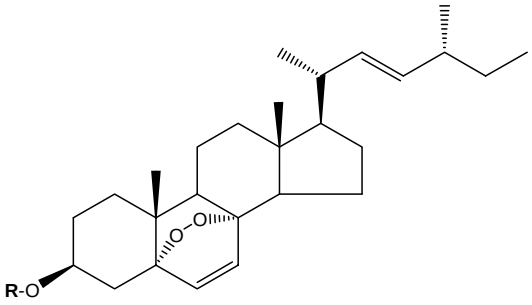
Li *et al.* (2002) showed observed resemblance and pharmacological efficacy of nucleoside and polysaccharide contents in fruiting body and caterpillar host by capillary electrophoresis (CE) and fast performance liquid chromatography (FPLC) analysis. It is noteworthy that the CE profile of the dry naive worm without *Cordyceps* invasion was completely different. Natural *O. sinensis* contains more than 7.99% free mannitol, whereas cultured *O. sinensis* contained less than 5.83% mannitol (Guan *et al.* 2010). Zhu *et al.* (1998a) classified seven classes of chemical constituents in natural and mycelium fermentation of *O. sinensis* as sterols, nucleoside compounds, saccharides and sugar derivatives, fatty acids and other organic acids, proteins, vitamins and inorganics. The constituents of *O. sinensis* are numerous and vary depending on the place of origin and type of strain used for mycelium culture. It is not our intention in this review to cover all compounds reported, but to summarize the major components (compounds **1-112**). Several characteristic compounds have been reported from *O. sinensis* (Fig. 2).



R

H (**1**) ergosterol

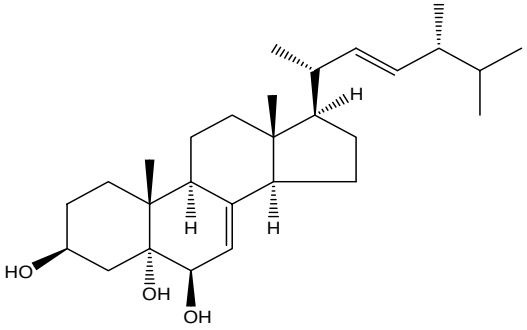
Glu (**2**) ergosteryl-3-*O*-*â*-D-glucopyranoside



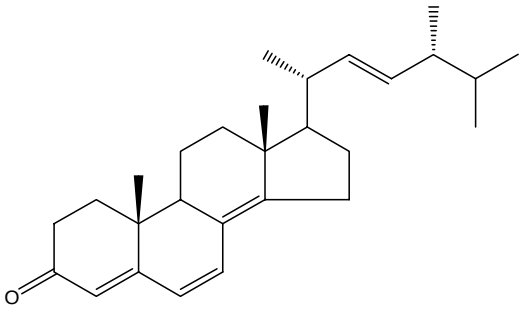
R

H (**3**) ergosterol peroxide [5*á*,8*á*-epidioxy-24(*R*)-methylcholesta-6,22-diene-3*â*-ol]

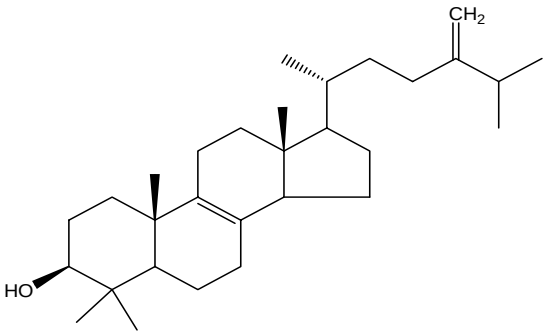
Glu (**4**) 5*á*,8*á*-epidioxy-24(*R*)-methylcholesta-6,22-dien-3*â*-*O*-*â*-D-glucopyranoside



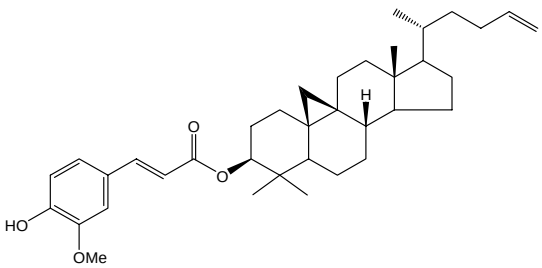
(**5**) cerevisterol (24*R*)-ergosta-7,22-diene-3*â*,5*á*,6*â*-triol



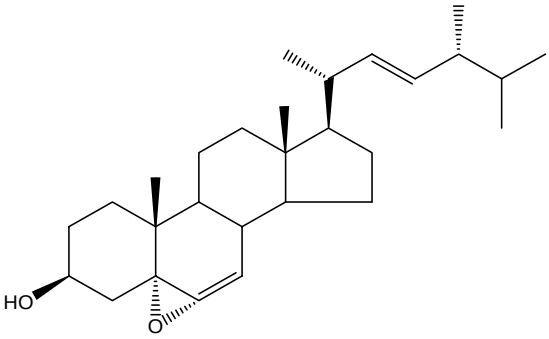
(**6**) ergosta-4,6,8(14),22-tetraen-3-one



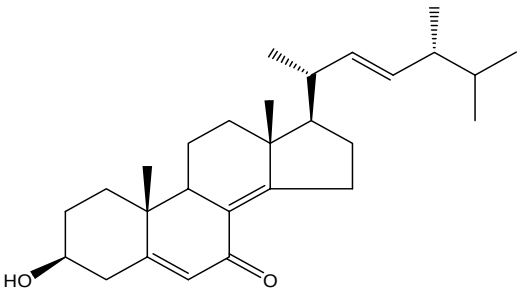
(**7**) 4,4-dimethyl-5*á*-ergosta-8,24(28)-dien-3*â*-ol



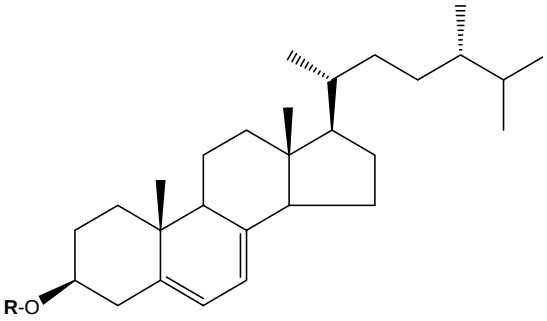
(**8**) 3-*O*-ferulylcycloartenol



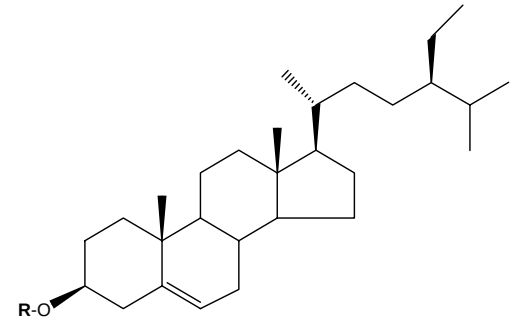
(**9**) 5*á*,6*á*-epoxy-24(*R*)-methylcholesta-7,22-dien-3*â*-ol



(**10**) ergosta-5,8(14),22-trien-7-one, 3*â*-ol [H1-A]



(**11**) 22,23-dihydroergosteryl-3-O-*â*-D-glucopyranoside

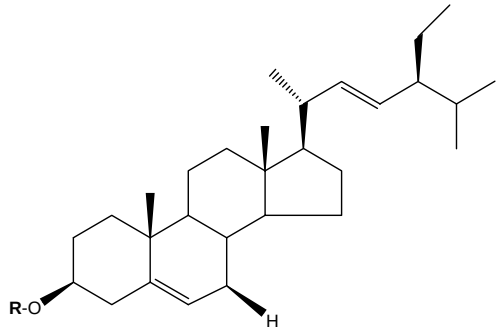


R

H (**12**) *â*-sitosterol

OAc (**13**) *â*-sitosterol 3-*O*-acetate

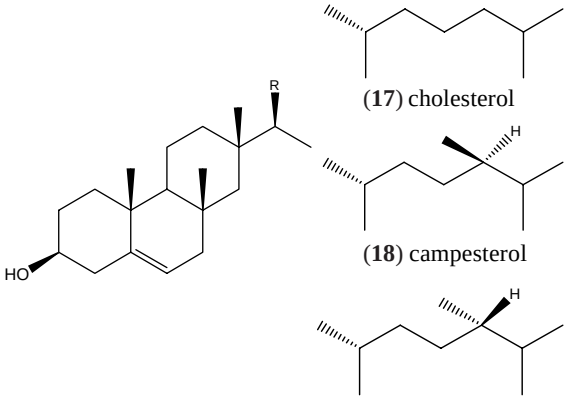
Glu (**14**) daucosterol



R

H (**15**) stigmasterol

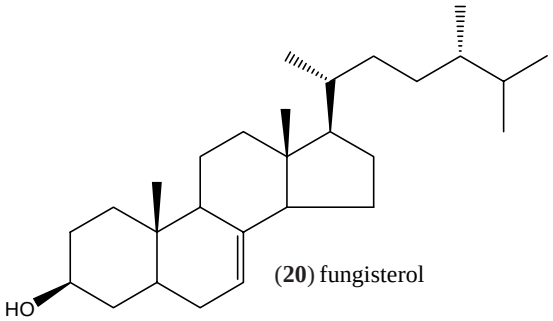
OAc (**16**) stigmasterol 3-*O*-acetate



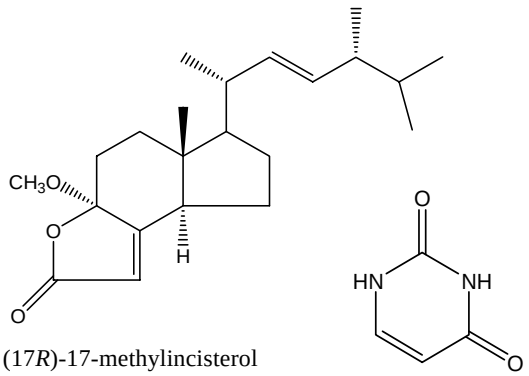
(**17**) cholesterol

(**18**) campesterol

(**19**) dihydrobrassicasterol (Δ^5-ergosterol)

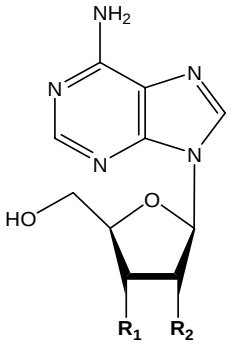


(**20**) fungisterol

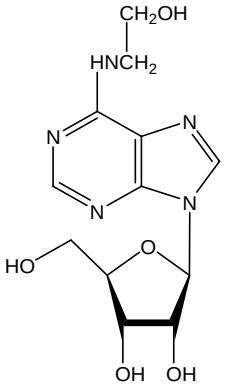


(**21**) (17*R*)-17-methylincisterol

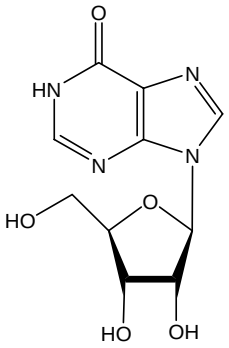
(**22**) uracil



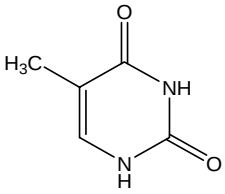
R_1	R_2	
OH	OH	(**26**) adenosine
H	OH	(**27**) cordycepin
H	H	(**28**) dideoxyadenosine



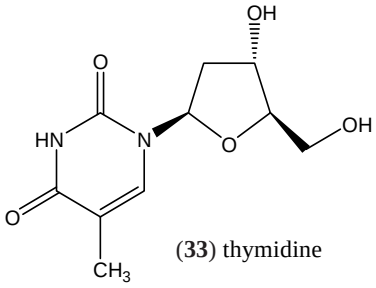
(**29**) N^6-(2-hydroxyethyl) adenosine



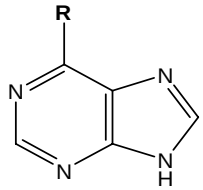
(**30**) inosine



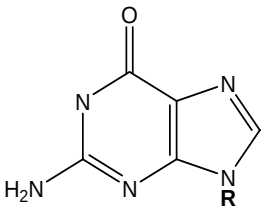
(**32**) thymine



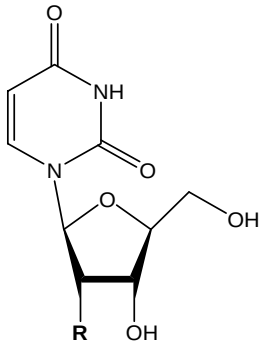
(**33**) thymidine



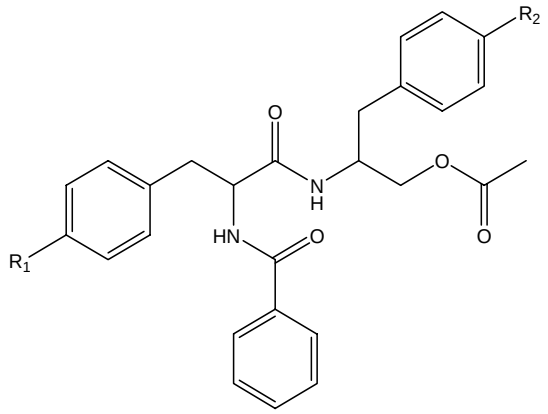
R	
NH_2	(**23**) adenine
OH	(**25**) hypoxanthine



R	
NH	(**24**) guanine
Ribose	(**31**) guanosine



R	
OH	(**34**) uridine
H	(**35**) deoxyuridine

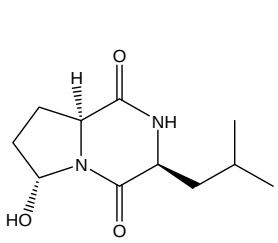


R_1 R_2

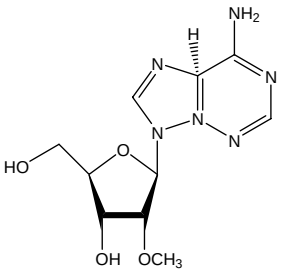
OH H (**36**) cordyceamide A [*N*-benzoyl-L-tyrosinyl-L-phenylalaninol acetate]

OH OH (**37**) cordyceamide B [*N*-benzoyl-L-tyrosinyl-L-*p*-hydroxyphenylalaninol acetate]

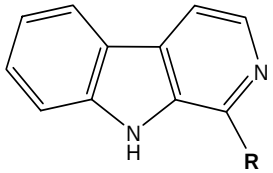
H H (**38**) aurantiamide acetate



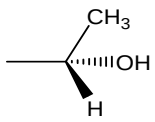
(**39**) cordysinin A



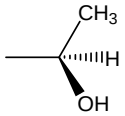
(**40**) cordysinin B



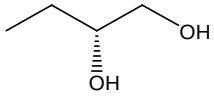
R



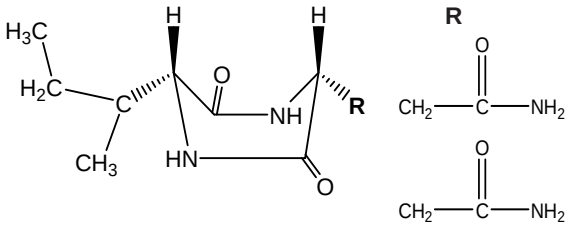
(**41**) cordysinin C



(**42**) cordysinin D

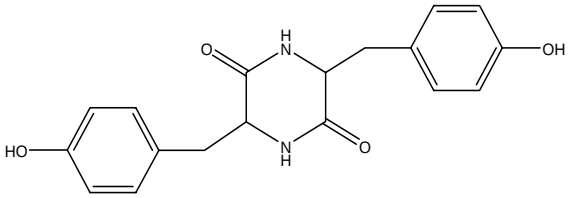


(**43**) cordysinin E

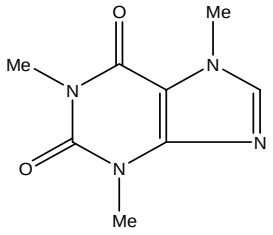


(**44**) cordycedipeptide A [3-acetamino-6-isobutyl-2,5-dioxopiperazine]

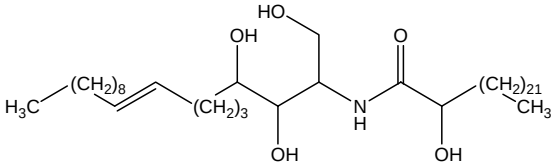
(**45**) 3-isopropyl-6-isobutyl-2,5-dioxopiperazine



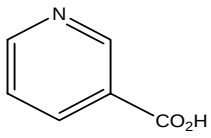
(**46**) 3,6-di(4-hydroxy)benzyl-2,5-dioxopiperazine



(**47**) caffeine



(**48**) *N*-(2'-hydroxy-tetracosanoyl)-2-amino-1,3,4-trihydroxy-octadec-8E-ene



(**49**) 2-nicotinic acid

Fig. 2. Chemical structures of characteristic compounds (**1**-**49**) from *O. sinensis*

Some new compounds recently reported from mycelium of *O. sinensis* are cordycedipeptide A [3-acetamino-6-isobutyl-2,5-dioxopiperazine, 3-isopropyl-6-isobutyl-2,5-dioxopiperazine 3,6-di(4-hydroxy) benzyl-2,5-dioxopiperazine] (**44**) (Jia *et al.* 2005), cordyceamides A and B (Jia *et at.* 2009) and cordysinins A-E (**39-43**) (Yang *et al.* 2011). Several polysaccharides have been isolated from *O. sinensis* (Zhong *et al.* 2009, Wu *et al.* 2006). Further, 51 volatile compounds (**V1-V51**) were characterized from the mycelia of *O. sinensis* (Yu *et al.* 2012). Among them phenols, acids and alkanes were found as the major classes of compounds. Among the total volatiles, butylated hydroxytoluene (BHT) (**V33**) was it is noteworthy that the most abundant volatile compound 47.38% and 46.12% in solid state media and submerged fermentation, respectively. There is very few report of presence of BHT in natural source (Babu & Wu 2008). Phosphonic acid (**V34**) was the second abundant compounds and accounted for 24.96% and 7.01% in mycelia cultured by submerged fermentation and mycelia cultured by solid-state media, respectively.

Pharmacological reports

In traditional medicine practices, the therapeutic effects are due to contribution of multiple components, not only the major ones. There exist extensive reviews on clinical aspects of *O. sinensis* (Zhu *et al.* 1998a,b, Holliday 2008, Li 2009). Here we have dealt with an overview of therapeutic values of major compounds present in *O. sinensis* and their pharmacological significances**.**

Cytotoxicity

There are reports of water, ethanol extract and polysaccharide fractions of *O. sinensis* attributing immune response rather than direct cytotoxicity (Wasser 2002, Nakamura *et al.* 2003, Yoshida *et al.* 1989). Zhao *et al.* (2011) reported ergosta-4,6,8(14),22-tetraen-3-one (**6**) induces G2/M cell cycle arrest and apoptosis in HepG2 cells in a caspase-dependent manner. Further the interference of altered nucleoside like cordycepin (**27**) can have a role in DNA repair mechanism for antitumor response (Holliday & Cleaver 2008). Jia *et al.* (2005, 2009) reported cordyceamides A (**36**) and B (**37**) as well as cordycedipeptide A (**44**) as cytotoxic against L-929, A375 and Hela IC50. Cordycedipeptide A (**44**) showed significant IC_{50} value of 6.30 mg/ml (L-929), 9.16 mg/ml (A375). Moreover, the glycosylated form of ergosterol peroxide (**4**) as a greater inhibitor to the proliferation of K562, Jurkat, WM-1341, HL-60 and RPMI-8226 tumor cell lines by 10 to 40% at 10 ìg/ml (Bok *et al.* 1999). The presence of above mentioned potential cytotoxic compounds might be responsible for anticancer property of *O. sinensis*.

Anti-inflammation

There are several reports of anti inflammatory property from extract of *O. sinensis* (Rao *et al.* 2007, Shahed *et al.* 2001, Kuo *et al.* 2001, Diniz *et al.* 2003, Jordan & Lee 2008). Kim *et al.* (2006) reported that cordycepin (**27**) markedly inhibited the phosphorylation of Akt and p38 in dose-dependent manners in LPS-activated macrophage. It suppressed tumor necrosis factor (TNF-á) expression, IêB alpha phosphorylation, and translocation of nuclear factor-êB (NF-êB). The expressions of cycloxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) were significantly decreased in RAW 264.7 cell by cordycepin (**27**). These results suggest that cordycepin (**27**) inhibits the production of NO production by down-regulation of iNOS and COX-2 gene expression via the suppression of NF-êB activation, Akt and p38 phosphorylation. Further, Yang *et al.* (2011) reported ergosterol-3-*O*-*â*-D-glucopyranoside (**2**) displayed the most significant inhibition of superoxide anion generation and elastase release with IC_{50} values of 5.42 ± 0.50 and 5.62 ± 0.37 ìM, respectively. They also reported 1-(5-hydroxymethyl-2-furyl)-*â*-carboline (perlolyrine) (**62**) displayed the most significant inhibition of superoxide anion generation and elastase release with IC_{50} values of 0.45 ± 0.15 and 1.68 ±0.32 ìM, respectively. Thus, cordycepin (**27**), ergosterol-3-*O*-*â*-D-glucopyranoside (**2**) and perlolyrine (**62**) may have provide a significant role in treatment of inflammation-associated disorders.

Antioxidant

Xiao *et al.* (2012) reported cordycepin (**27**) could ameliorate albumin-induced EMT of HK2 cells by decreasing NADPH oxidase activity and inhibiting reactive Oxygen Species (ROS) production. A heteropolysaccharide, CS-PS have been reported for its effect on immunity activity in mice treated by ionizing radiation, through reducing oxidative injury and modulating the secretion of cytokine IL-4, IL-5 and IL-17 (Zhang *et al.* 2011). Wang *et al.* (2012) reported cordymin (**68**) significantly enhanced the defense mechanism against cerebral ischemia by increasing antioxidants activity related to lesion pathogenesis leading the brain recover from ischemic injury. Yang *et al.* (2011) observed ergosteryl-3-*O*-*â*-

D-glucopyranoside (**2**) and perlolyrine (**62**) inhibited superoxide anion generation and elastase release in FMLP/CB-activated human neutrophils. They reported that the most significant inhibition toward superoxide anion generation and elastase release with IC_{50} values of 0.45 ± 0.15 and 1.68 ± 0.32 ìM, respectively were demonstrated by perlolyrine. Further, compound 3',4',7-trihydroxyisoflavone (**96**) displayed significant scavenging of DPPH free radicals with IC_{50} values of 31.97 ìM. Therefore, antioxidant activity may be attributed to the presence of ergosteryl-3-*O*-*â*-D-glucopyranoside (**2**), perlolyrine (**62**) and 3',*4*',7—trihydroxyisoflavone (**96**). Polysaccharide CPS1 (**107**) provided scavenging effects on the hydroxyl radicals, the reducing power, Fe^{2+}-chelating activity, scavenging effect on superoxide radicals, antioxidant activity and showed a connection between antioxidant activity and reparation of renal failure (Wang *et al.* 2009). The polysaccharide CSP-1 (**106**) is known for significant protection against the free radical-induced neuronal cell toxicity by attenuating the changes of glutathione peroxidase and superoxide dismutase activities in H_2O_2-treated cells in a dose-dependent manner (Li *et al.* 2003).

Immunomodulating effect

The renal failure is one of the major human health issues and improvement of renal function by use of *O. sinensis* is reported (Guan *et al.* 1992). Xu *et al.* (1995) reported immunomodulating effects of *O. sinensis* in kidney transplanted patients. The lymphoproliferative response, natural killer (NK) cell activity, and phytohemagglutinin (PHA) stimulated interleukin-2 (IL-2) and tumor necrosis factor-alpha (TNF-alpha) production on human mononuclear cells (HMNC) (Kuo *et al.* 1996). Lin *et al.* (1999) observed H1-A (**10**) can suppress the activated human mesangial cells HMC and alleviate IgAN (Berger's disease) with clinical and histological improvement. Ergosterol (**1**) is well known as diuretic bioactive compound with excellent efficacy (Zhao 2009). Ding *et al.* (2011) reported lowering of serum level of IL-10 in kidney transplanted patient, which can be beneficial in long term treatment requiring cyclosporin. Lin (2002) reported (24*R*)-ergosta-7,22-dien-3*â*,5á,6â-triol (**5**) improved kidney function in renal diseases, including IgA nephritis, autoimmune nephritis, and lupus nephritis by inhibiting IL-2 formation by monocyte and proliferation of mesangial cells and lymph node.

Hyperglycemia

Guo and Zhang (1995) reported potentiality of *O. sinensis* in blood sugar regulation. Kiho *et al.* (1996) reported CS-F30 (**101**) increased the activities of hepatic glucokinase, hexokinase and glucose-6-phosphate dehydrogenase and decreased glycogen content in the liver. Furthermore, Kiho *et al.* (1999) reported CS-F10 (**102**) significantly increased the activity of hepatic glucokinase in streptozotocin (STZ) -induced diabetic mice leading to reduced hepatic glucose output. Li *et al.* (2006) reported CSP-1 (**106**) a strong antioxidant showed with hypoglycemic properties increased circulating insulin level in diabetic animals, suggested that CSP-1 (**106**) may stimulate pancreatic release of insulin and/or reduce insulin metabolism. Yun *et al.* (2003) reported cordycepin (**27**) can reduced blood glucose level by 35.5% (acarbose, 37.2%) in STZ induced diabetic mice and elaborated that it can be promising new drug as an anti-hyperglycemic agent without defects of immune responses and the other side effects.

Asthemia

O. sinensis is known for its beneficial effect on various lung ailments (Holliday and Cleaver 2008) as well as in severe acute respiratory syndrome (SARS) (Chen *et al.*, 2012). Yue *et al.* (2008) reported cordycepin and adenosine stimulated ion transport in a dose-dependent manner in Calu-3 monolayers and which may be responsible for anion movement from the basolateral to apical compartments in the airway epithelia. Basolateral Na^+-K^+-$2Cl^-$ cotransporter and apical cAMP-dependent cystic fibrosis transmembrane conductance regulator Cl^- channel are involved in the process leading to pharmacological effect on the respiratory tract. Lin *et al.* (2004) reported use of (24*R*)-ergosta-7,22-diene-3*â*,5*á*,6*â*-triol (**5**) for preventing and treating bronchial hyper responsiveness and acute asthma attack and improving pulmonary function.

Heart ailment (arrhythmias, cardiac arrest)

Jordan *et al.* (2008) reported use of *O sinensis* to reduce acute and chronic rejection associated with cardiac transplantation. The use of *O. sinensis* extract in conjunction with a sub-therapeutic dose of cyclosporine significantly reduce $CD8^+$ T cell activity and can suppress acute rejection, ablates allograft vasculopathy. The occurrence of butylated hydroxytouline (**V33**) (Yu *et al.* 2012) in a significant amount can be accounted for its role in

hypercholesterolemia and atherosclerosis (Jilal & Devraj 1996).

Hepatoprotection

There are several reports of the protective effect of *O. sinensis* extract for liver cirrhosis (Wang *et al.* 2008), hepatitis B (Zhou 1990, Wang *et al.* 2012), non-alcoholic fatty liver (Yang *et al.* 2007). Lu *et al.* (2011) reported polysaccharides from *O. sinensis* can decrease superoxide dismutase (SOD) activity and ameliorate local necrosis in liver.

Hyperlipidemia / Hypercholesterolemia

Kiho *et al.* (1996) reported CS-F30 (**101**) lowered the plasma triglyceride level and cholesterol level in mice. Koh *et al.* (2003) showed hot-water extract of *O. sinensis* can change very-low-density lipoprotein plus low-density lipoprotein (VLDL+LDL) and consequently decreased the atherogenic index and decreased blood serum cholesterol levels. The investigation of Yu *et al.* (2012) showed the presence of butylated hydroxytouline (BHT) 47.5% and 42.22% in mycelia culture by solid media and submerged fermentation, respectively. BHT (**V33**) is well known for inhibition of LDL-oxidation (Xiu *et al.* 1994), with IC_{50} value of 2.1 µM (Shrestha *et al.* 2011). Presence of BHT in a significant amount can be reason of *O. sinensis* efficacy in treating hyperlipidemia and hypercholesterolemia (Jilal & Devraj 1996). The study of Kim (2010) showed strong inhibition of cholesterol esterase with an IC_{50} value of 12.7 ìg/mL by heteropolysaccharide PS-A (**109**), suggesting its use as a potential agent for control of hypercholesterolemia.

Sexual dysfunction

O. sinensis a trusted aphrodisiac, which enhances of libido and fertility in both sexes. Huang et al. (2004) reported steroidogenic enzyme expression in human granulosa-lutein cells (GLC). Ulibarri and Yahr (1997) observed cordycepin (**27**) treatment lead three times increased in lordosis quotients (LQs) and ovulation than in control. Further, Pao *et al.* (2012) reported cordycepin activated the phospholipase C/protein kinase C (PLC/PKC), but not PKA and PI3K, pathway to induce MA-10 cell steroidogenesis. Furthermore, Lew *et al.* (2011) showed stimulatory effects of cordycepin (**27**) on mouse leydig cell steroidognenesis. Nitric oxide (NO) has been well characterized as a mediator of inhibitory non-adrenergic non-cholinergic (NANC) neurotransmission in many organs including the urogenital system (Rand & Li 1995). There are a series of reactions involved in the erection of muscles, in which NO is utilized. NO is the principal vasodilator, it activates guanylyl cyclase, present in the cell membrane, resulting in an increase in the level of cyclic guanosine mono phosphate (cGMP) leading to the relaxation of smooth muscles of the corpora cavernousa allowing inflow of blood leading to tumescence in both sexes (Burnett *et al.* 1992, Cellek & Moncada 1998, Kim *et al.* 2003, Bansal *et al.* 2010). *b*-Sitosterol (**12**) is known for reinforcing the cell viability as well as increase in NO production significantly (Zhang *et al.* 2011) and D-mannitol (**112**) also facilitates NO production (Ohkuma *et al.* 1998) and both are major components in *O. sinensis* (Yu *et al.* 2012), which can be the possible cause of arousal or treatment for erectile dysfunction by *O. sinensis*.

Immunoresponse

Holliday and Cleaver (2008) mentioned *O. sinensis* as an option for antiretroviral drug. Further, Cheung *et al.* (2009) reported an exo-polysaccharide, cordysinocan (**108**) induced the cell proliferation and the secretion of interleukin-2, interleukin-6 and interleukin-8 in cultured T-lymphocytes. Furthermore, the phosphorylation of extracellular signal-regulated kinases (ERK) was induced transiently by its treatment and increased the phagocytosis activity and the enzymatic activity of acid phosphatase which verify the important its role of cordysinocan (**108**) in triggering immune responses.

The unique assemblage of sterols, nucleosides and polysaccharides as major constituents in *O. sinensis* are responsible for its widely acclaimed therapeutic value. The standardization of *O. sinensis* in various regions of Nepal and identification of most potential sites is needed. Further research for culturing *O. sinensis* in selected wild habitat with the artificial infection as well as adjustment of temperature, humidity and reduction of natural predators of *Hepialus* larva is needed to ensure sustainable harvest as well as economic benefit. Winkler (2009) reported that revenue of District forest office in Dolpo showed the steady rise in collection, 2006 (148 kg), 2007 (241 kg), 2008 (773 kg) and 2009 (872 kg). However, drastic decline collection this year should be taken as a wakeup call for urgent need management of collection from nature (Taggart 2012). Extensive research on metabolites from wild *O. sinensis* is still awaited as researches are focused on mycelium culture due to relatively high cost of wild specimen.

Acknowledgements

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