Fitoterapia 81 (2010) 961–968

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Review

Medicinal uses of the mushroom *Cordyceps militaris*: Current state and prospects

Shonkor Kumar Das, Mina Masuda, Akihiko Sakurai *, Mikio Sakakibara

Department of Applied Chemistry and Biotechnology, Graduate School of Engineering, University of Fukui, 3-9-1 Bunkyo, Fukui 910-8507, Japan

A R T I C L E I N F O

Article history:
Received 12 July 2010
Accepted 13 July 2010
Available online 19 July 2010

Keywords:
Medicinal uses
Culture techniques
Current state
Prospects
Pharmacognosy
Cordyceps militaris

A B S T R A C T

Cordyceps militaris is a potential harbour of bio-metabolites for herbal drugs and evidences are available about its applications for revitalization of various systems of the body from ancient times. Amongst all the species, *C. militaris* is considered as the oldest source of some useful chemical constituents. Besides their popular applications for tonic medicine by the all stairs of the community, the constituents of *C. militaris* are now used extensively in modern systems of medicine. The current survey records the mysterious potentials of *C. militaris* are boosting up the present herbal treatments, as well as gearing up the green pharmacy revolution, in order to create a friendly environment with reasonable safety. Evidence showed that the active principles of *C. militaris* are beneficial to act as pro-sexual, anti-inflammatory, anti-oxidant/anti-aging, anti-tumour/anti-cancer/anti-leukemic, anti-proliferative, anti-metastatic, immunomodulatory, anti-microbial, anti-bacterial, anti-viral, anti-fungal, anti-protozoal, insecticidal, larvicidal, anti-fibrotic, steroidogenic, hypoglacaemic, hypolipidaemic, anti-angiogenetic, anti-diabetic, anti-HIV, anti-malarial, anti-fatigue, neuroprotective, liver-protective, reno-protective as well as pneumo-protective, let alone their other synergistic activities, which let it be marketable in the western countries as over-the-counter medicine. A number of culture techniques for this mushroom have been noticed, for example, storage/stock culture, pre-culture, popular/indigenous culture (spawn culture, husked rice culture and saw dust culture) and, special/laboratory culture (shaking culture, submerged culture, surface liquid culture and continuous/repeated batch culture). The prospects for herbal biotechnology regarding drug discovery using *C. militaris* delivering what it has promised are high, as the technology is now extremely more powerful than before. This study chiefly highlights the medicinal uses of the mushroom *C. militaris* including its culture techniques, also aiming to draw sufficient attention of the researchers to the frontier research needs in this context.

Contents

* Corresponding author. Tel.: +81 776 27 8924; fax: +81 776 27 8747.
E-mail addresses: skdas76@yahoo.com (S.K. Das), mmasuda@u-fukui.ac.jp (M. Masuda), a-sakura@u-fukui.ac.jp (A. Sakurai), m-sakaki@u-fukui.ac.jp (M. Sakakibara).

0367-326X/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.fitote.2010.07.010

1. Introduction

Cordyceps genus, the name given to the fungi on insects and its existence has been known since 2000 B.C. The medicinal mushroom (*Cordyceps* species) is an abundant source of useful natural products with various biological activities [1]. One of the most important traditional Chinese medicines, *Cordyceps militaris* (an entomopathogenic fungus) which belonging to the class *Ascomycetes*, has been used extensively as a crude drug and a folk tonic food in East Asia [2]. It contains many kinds of active components (such as cordycepin, polysaccharides, ergosterol, mannitol, etc.), and due to its various physiological activities, it is now used for multiple medicinal purposes [3–5]. In the wide implications of animal fungi parasitic to the insects, more than 350 types have been discovered until now. At present, the elaborative researches about its elements are being done in the universities and other research institutions; this has increased greatly its medicinal value. There are over 2500 mushroom varieties grown in the world today [6]. It is estimated that more than 10 million metric tons of edible and medicinal mushrooms were produced last year in various countries [7]. That is why, recently mushroom has received significant attention from medical and pharmacological researchers as a rich source of biologically active compounds [8].

Nowadays, medicinal herbs are becoming increasingly popular and important in the public and scientific communities. In contrast to their regulated status in China and other countries, herbal medicines are regarded as dietary supplements in the United States and the use of herbal supplements in the United States is steadily growing and raises concerns about safety, efficacy, and how they affect safe patient care [9]. Elsewhere, the increasing use of herbal products worldwide and the growth of the herbal product industry have led to increasing concern regarding their safety [10]. WHO estimated that about three-quarters of the world's population currently use herbs and other forms of traditional medicines to treat their diseases [11] including breast cancer (12%) [12], liver disease (21%) [13], HIV (22%) [14], asthma (24%) [15] and rheumatological disorders (26%) [16]. The acceptance and recognition of herbal medicine has been in part, due to the acknowledgement of the value of traditional and indigenous pharmacopoeias, the incorporation of some medicines derived from these sources into pharmaceuticals [17,18], the need to make health care affordable for all and the perception that pharmaceutical drugs are increasingly over prescribed, expensive and even dangerous. Another important perception fomenting this interest is that natural remedies are somehow safer and more efficacious with fewer side effects than remedies that are pharmaceutically derived [19,20].

Simultaneously, the development of modern chemistry permitted the isolation of chemicals from medicinal herbs that have served as drugs or starting materials for the synthesis of many important drugs used today. Many more modern drugs have been synthesized as a result of knowledge gained from studies of mechanisms of actions of chemicals first isolated from medicinal herbs including medicinal mushrooms. Thus, medicinal herbs have played a major role in the development of modern medicine and continue to be widely used in their original form [21].

The main active constituent of *C. militaris* fruiting bodies is cordycepin, which was first extracted from *C. militaris* and then found to be present in *Cordyceps sinensis* [22] and *Cordyceps kyushuensis* [23]. The important bioactive compound cordycepin (3′-deoxyadenosine, , m.p. 225 °C, α D-47 °C), a nucleoside analogue [22,24], is considered as a nucleic acid antibiotic that might inhibit canceration of cells contributing to the normalization of cancer cells as one of constituents of gene DNA [22]. The recent studies have demonstrated that the extracts of *C. militaris* have multiple pharmacological actions, such as, inhibition of human glomerular mesangial cell proliferation [25], anti-fibrotic [26], anti-angiogenetic [27], improvement of insulin resistance and insulin secretion [28], anti-inflammatory [29], and growth inhibition of U937 leukemia cells [30]. Besides, it is also reported that cordycepin itself acts as an anti-tumour, anti-proliferative, anti-metastatic, insecticidal and anti-bacterial compound [4]. Therefore, the medicinal mushroom *C. militaris* (Fig. 1) is one of the most important candidates that may be used as the herbal medicinal bases in the future for the welfare of mankind.

The mycological data of *C. militaris* and popular names of *Cordyceps* are given in Tables 1 and 2, respectively.

2. Culture information

2.1. Culture type

2.1.1. Storage culture/stock culture

The stock culture is usually stored on a 39-g/l PDA slant at 5 °C.

2.1.2. Pre-culture (slant and plate culture)

Both of the slant and plate culture are prepared on 39-g/l PDA and, usually stored at 25 °C.

Fig. 1. *Cordyceps militaris* (www.jscr.jp).

2.1.3. Popular/indigenous culture [3]

There are a number of indigenous/popular culture techniques which vary in places, environment and, aim of the culture considered. These are discussed below.

2.1.3.1. Spawn production. Usually glass beads are added to the potato glucose medium, which is then sterilized in an autoclave at 121 °C for 20 min. After cooling, the medium is inoculated with several pieces of stock culture. After stationary culturing at 25 °C for a week, the hyphae are dispersed by shaking the medium once a day. When the fungus proliferated, it is inoculated into bead-free potato glucose medium and cultured therein for 5 days to produce a seed culture.

In the case of solid medium, a small amount of actively growing hyphae from the slant is used to give a seed culture. Subsequently, some amount of the sawdust can be added to the medium to enlarge the culture scale.

2.1.3.2. Husked rice culture. The husked rice medium or the wheat medium is inoculated with the liquid spawn. The inoculation contributes to the supplement of moisture and addition of auxiliary nutrients. After culturing at 25 °C for about 20 days, the fungus proliferates all over the medium and then it is aged for about 1 month.

2.1.3.3. Sawdust culture. Two kilograms of solid medium is inoculated with one spoonful of the seed culture that had been pre-cultured in the sawdust. After culturing at 25 °C for about 20 days, the fungus proliferates all over the medium. Then, it is cultured in the dark at 20–25 °C for about 6 months. Thus, knot like fruiting bodies are formed.

Table 1
Mycological data of *Cordyceps militaris*.

Kingdom	Fungi
Phylum	Ascomycoa
Sub-phylum	Ascomycotina
Class	Ascomycetes/Pyrenomycetes
Order	Hypocreales
Family	Clavicipataceae
Genus	*Cordyceps*
Species	*Cordyceps militaris*

Table 2
Popular names of *Cordyceps*.

Common name	Caterpillar fungus, *Cordyceps*, Cetepiller mushroom
Latin/English name	*Cordyceps militaris*, *Cordyceps* mushroom, Deer fungus, Caterpillar fungus
Chinese name	Dong Chong Xia Cao, Summer grass-winter worm, Hia tsao tong tchong
Japanese name	Tochukaso/Tochukasu, Totsu kasu
Korean name	Tong ch'ug ha ch'o
Nepali name	Yarsagumba, Jeebanbuti, Sanjivani, Kiraghans
Tibetian name	Yarchakunbu
Other names	Chong cao, Dong chong cao, Aweto

2.1.4. Special culture/laboratory culture

These are the specialized culture techniques used for the culture of *C. militaris*, depending on the target of production and conditions provided. These are discussed below.

2.1.4.1. Shaking culture. Pre-culture is the same as described above and, the mycelia of *C. militaris* are transferred to the seed culture medium by punching out about 5 mm^2 of the plate with a sterilized cutter. The seed culture is grown in a 250 or 500 ml shake flask containing 50–100 ml of liquid medium and incubated at 25 °C on a rotary shaker (50–150 rpm, depends on culture aim) for 5–7 days, or to the day when the carbon source becomes zero. Shaking culture is also one kind of submerged culture and all the conditions of this technique depend on the research aim, time allocated and environments needed.

2.1.4.2. Submerged culture. In this process, the organism is grown in a liquid medium, which is vigorously aerated and agitated in large tanks called fermentors. The fermentor could be either an open tank or a closed tank and may be a batch type or a continuous type and are generally made of non-corrosive type of metal or glass lined or of wood.

2.1.4.3. Surface liquid culture. Prior to the surface liquid culture, the active PDA slant of the mycelia is prepared by culturing for 8 days at 25 °C, and then the seed culture transferred from the active slant is allowed to grow on PDA medium in a plate for 13–20 days at 25 °C (depending on the type of strain, as for control 13 days, but for mutant 20 days usually). The inoculum is prepared by punching out 1 cm disk of the PDA plate culture using a sterilized cylindrical cutter. The surface liquid culture is started by inoculating the inoculum (seed disk) into a 500 ml culture bottle (size varies with the research aim), which has an 8.5 cm diameter and 14.0 cm height with a bottleneck whose diameter and height are 4.5 and 4.0 cm, respectively. The working volume of the surface liquid culture medium is 100 ml for each bottle, and the bottleneck is fitted with a cotton plug during the culture. The prepared bottles are placed in an incubator maintained at 25 ± 1 °C. The sampling can be done at various intervals through the sampling port for analysis. Just before the sampling, it is better to agitate the medium gently for 5 seconds by a magnetic stirrer. Then, the filtrate can be analyzed for

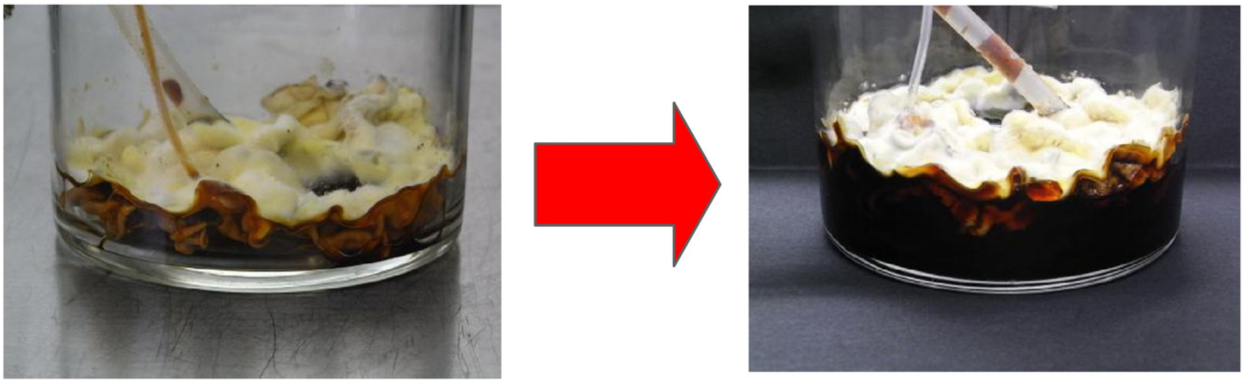

Fig. 2. Replenishment of medium in a repeated batch culture.

targeted materials (for example, cordycepin, glucose, pH, etc.) [31].

2.1.4.4. Continuous culture or repeated batch culture. It is well known that the greatest gain in productivity can be achieved by a vicious culture system rather than one cycle cell culture technique, as the fungal/mushroom cells grow substantially slower in a shuttle culture system, due to lack of required nutrition supply at the end of the each culture cycle. Therefore, the conventional culture system is limited to time duration accompanied by a low productivity, whereas, the repeated batch culture is independent of time duration, may provide better productivity.

A repeated batch culture technique is one in which un-useful medium is removed at the end of each batch and the displaced medium is replenished with the fresh medium (Fig. 2). The constant addition of a fresh medium and elimination of an un-useful one provides the cells with the environment they require actually to achieve higher productivity. Therefore, a repeated batch technique using surface liquid culture is considered as the best one for obtaining the highest productivity.

2.2. Culture media [3,31,32]

A number of solid and liquid media are used to culture *C. militaris*. Solid media may be beech wood meal, rice bran, wheat bran, husked rice, wheat grains and, culture bag or bottles can be used for the purpose. Several liquid media are also used depending on the aim of culture and, on the basis of concentration of the medium components those may be basal, enriched and enriched media with an additive. Basal media having the minimal medium components for the growth of the fungus and enriched medium having higher/optimum medium concentration aiming to attain a peak production. The suitable additives those could influence its growth and, thus could increase production are usually used for this purpose.

To our knowledge, it was revealed that a basal medium has a lower production [33] and, the optimized media for *C. militaris* mutant and control each showed higher productions as 6.84 and 2.45 g/l, respectively [34]. A further higher production was also attained using *C. militaris* mutant using adenosine (6 g/l) as an additive as 8.57 g/l and, this is the highest production of cordycepin reported until today [35]. A repeated batch culture also showed a higher productivity than those of the control, prospective for pharmaceutical uses. A table having a basal, optimized and optimized medium with additive is given below (Table 3).

3. Chemical constituents [4]

A variety of compounds have been purified and their structures were elucidated. Cordycepin (3′-deoxyadenosine, $C_{10}H_{13}N_5O_3$), 3′-amino-3′-deoxyadenosine ($C_{10}H_{14}N_6O_3$), homocitrullyl aminoadenosine ($C_{17}H_{27}N_9O_5$), adenine ($C_5H_5N_5$), cordycepic acid ($C_6H_{14}O_6$) and D-mannitol ($C_6H_{14}O_6$) have been reported from *Cordyceps* species [36–40]. Nucleosides and their bases are determined in *Cordyceps* [41–43]. Determination of adenosine ($C_{10}H_{13}N_5O_4$) and 3′-deoxyadenosine (cordycepin) using HPLC method also reported by some researchers [44,45]. Besides these, ergosterol ($C_{28}H_{44}O$) in *Cordyceps* can be determined by HPLC method [41,46]. Ophiocordin ($C_{28}H_{26}N_2O_{10}$), an anti-fungal antibiotic, is found in this *Cordyceps* [47,48]. Bioxanthracenes ($C_{34}H_{36}O_{10}$) were also isolated from *Cordyceps* [49,50]. In addition, cordyheptapeptide ($C_{49}H_{65}N_7O_8$), a novel cycloheptapeptide, was isolated from a strain of *Cordyceps* together with four known bioxanthracenes. There were only two previous reports on the isolation of cyclic peptides from this genus and these were *C. militaris* and *C. sinensis* [51]. Hypoxanthine ($C_5H_4N_4O$) was also separated along with adenine and cordycepin from *Cordyceps* [45]. Water-soluble crude polysaccharides were obtained from the fruiting bodies of cultured *C. militaris* by hot-water extraction followed by ethanol precipitation. These polysaccharides were successively purified by chromatography giving several fractions [52]. Finally, four exopolysaccharides with different molecular masses ranging from 50 kDa to 2260 kDa were also reported from *C. militaris* by several researchers [53–56]. Ten-membered macrolides ($C_{10}H_{14}O_4$), cephalosporolides C, E and F, cordycepin, pyridine-2, 6-dicarboxylic acid ($C_7H_5NO_4$) and 2-carboxymethyl-4-(3′-hydroxybutyl) furan were also reported from *C. militaris* [57]. In addition, a method for concurrent determination adenosine and cordycepin from *C. sinensis* and *C. militaris* also described [58]. In *Cordyceps* species, adenine, adenosine, guanosine ($C_{10}H_{13}N_5O_5$), uracil ($C_4H_4N_2O_2$), uridine ($C_9H_{12}N_2O_6$) and inosine ($C_{10}H_{12}N_4O_5$) can be determined by a capillary electrophoresis [59].

Table 3
Composition of different types of media for the mutant and control of *C. militaris*.

Components	Concentration (g/l)			
	Basal medium		Optimized media	
	Basal	Control	Mutant	Mutant + Adenosine
Nitrogen sources				
Yeast extract	7.5	72.5	93.8	93.8
Peptone	2.5			
Carbon source				
Glucose	20	62.6	86.2	86.2
Additive				
Adenosine				6
Others (diluted to 1/10 concentration of Vogel's medium) (Same for both mutant and the control)				
$NaOC(COOH)(CH_2COONa)_2\cdot 2H_2O$		0.28		
KH_2PO_4		0.5		
NH_4NO_3		0.2		
$MgSO_4\cdot 7H_2O$		0.02		
$CaCl_2\cdot 2H_2O$		0.01		
Citric acid		0.46×10^{-3}		
$ZnSO_4\cdot 7H_2O$		0.50×10^{-3}		
$Fe(NH_4)_2(SO_4)_2\cdot 6H_2O$		0.10×10^{-3}		
$CuSO_4\cdot 5H_2O$		0.025×10^{-3}		
H_3BO_3		5.0×10^{-6}		
$MnSO_4\cdot (4–5)H_2O$		5.0×10^{-6}		
$Na_2MoO_4\cdot 2H_2O$		5.0×10^{-6}		

Cicadapeptins I and II (peptides containing α-aminoisobutyric acid, $C_{50}H_{90}N_{10}O_{11}$) and myriocin ($C_{21}H_{39}NO_6$, a fungicide) were also reported in this species [60]. A glycoprotein with *N*-acetylgalactosamine ($C_8H_{15}NO_6$) was also isolated from *Cordyceps* [61]. An inhibitor of the prophenoloxidase activation was isolated from the culture filtrate of *C. militaris* and identified as pyridine-2, 6-dicarboxylic acid, also known as dipicolinic acid (DPA, $C_7H_5NO_4$) [62]. A lectin from *C. militaris* exhibited hemagglutination activity in mouse and rat erythrocytes, but not in human ABO erythrocytes [63].

The nucleic acid-related compounds obtained from *C. militaris* are shown in Table 4 [32].

4. Biological activities of *C. militaris* [5]

A number of valuable biological activities have been encountered for *C. militaris* by several authors (Table 5). It was revealed that most of biological activities more or less similar to those of *C. sinensis*.

5. Medicinal uses/clinical applications of *Cordyceps* including *C. militaris* [3,86]

C. sinensis was used more extensively than *C. militaris* and, their clinical applications are more or less similar. Although it is well known as a pro-sexual, anti-inflammatory and anti-cancer agent, it is presently using for many clinical cases like, insufficient pulmonary function, coughing, sputum, dizziness, memory failure, myodesopsia, vision failure, cold virus, in appetence, night sweat, pale face, pale lips, buzzing in the ears, toothache and loose teeth, insomnia and thirsty, cold or hot limbs, lumbago or pain in knees, nervous prostration, diabetes, night enuresis, sexual impotence, anemia and slow recovery from illness.

6. Current state, limitations and remedies

The natural *C. militaris* is expensive in the local market. In the present state, the production of cordycepin from the fruiting body of *C. militaris* is not likely to reach commercial levels due to some practical limitations [33]. For example, *C. militaris* is very scarce in nature due to the requirements of specific hosts and strict growth environments [34]. On the other hand, it is necessary to note that the chemical synthesis of cordycepin requires a complicated process resulting a lower productivity, moreover, a large volume of organic solvents those are harmful substances to the environment may be discharged [87,88]. The friendly production of cordycepin from the cultured mycelia of *C. militaris* in a

Table 4
Nucleic acid-related compounds obtained from *C. militaris*.

Contents	Extract from mycelia (mg)	Filtrate of medium (mg)
C. militaris NBRC 9787		
Mycelia	3.29×10^4	
Adenine	2.9	2.8
Guanine	18.5	175.2
Uracil	37.1	105.0
Adenosine	91.1	52.6
Guanosine	78.7	47.1
Urideine	106.8	81.1
Cordycepin	56.2	2.5×10^3
C. militaris G81-3		
Mycelia	3.87×10^4	
Adenine	1.6	5.1
Guanine	23.2	271.7
Uracil	29.2	185.2
Adenosine	45.4	132.0
Guanosine	41.3	97.8
Uridine	45.4	122.6
Cordycepin	129.7	4.5×10^3

All values were estimated on the basis of a working volume of 1 L.

Table 5
Biological activities of *Cordyceps militaris*.

Biological activity	References	Biological activity	References
Pro-sexual	Yu et al. [52]	Insecticidal	Mao and Zhong [74]
	Lin et al. [64]		Kim et al. [80]
Anti-inflammatory	Yu et al. [55]	Larvicidal	Kim et al. [80]
	Won and Park [29]	Anti-fibrotic	Nan et al. [26]
Anti-oxidant/anti-aging	Yu et al. [52]	Steroidogenic	Shih et al. [71]
	Chen et al. [65]	Hypoglacaemic	Choi et al. [28]
Anti-tumour/anti-acncer/anti-leukemic	Liu et al. [66]		Yu et al. [52]
	John and Adamson [67]	Hypolipidaemic	Yu et al. [55]
	Muller et al. [68]		Shen and Chen [81]
	Kodama et al. [69]	Anti-angiogenic	Yoo et al. [27]
	Penman et al. [70]	Anti-diabetic	Choi et al. [28]
Anti-proliferative	Liu et al. [66]	Anti-HIV	Mueller et al. [78]
Anti-metastatic	Liu et al. [66]	Anti-malarial	Sugar and McCaffrey [82]
	Shih et al. [71]	Anti-fatigue	Jung et al. [83]
Immunomodulatory	Lin and Chiang [72]		Mizuno [3]
	Sone et al. [73]	Neuroprotective	Ribeizo [84]
	Mao and Zhong [74]		Gu et al. [1]
	Shih et al. [71]	Liver-proctective	Jung et al. [83]
Anti-microbial	Park [75]		Yu et al. [52]
Anti-bacterial	Ahn et al. [76]		Won and Park [29]
Anti-viral	Lin and Chiang [72]		Yu et al. [55]
	Ortiz et al. [77]	Reno-protective	Zhao-Long et al. [25]
	Mueller et al. [78]		Wu et al. [85]
	Mao and Zhong [74]		Yu et al. [52]
Anti-fungal	Mao and Zhong [74]	Pneumo-protective	Yu et al. [52]
	Shih et al. [71]		
Anti-protozoal	Trigg et al. [79]		

large scale is currently an acute issue. Some experiments have already proven that the chemical components of natural and cultured *C. militaris* are similar [89,90].

It is well known that since its discovery as an ancient medicinal tonic, it was facing several limitations till today. Negligence, lack of adequate awareness (lack of extension technology) and systematic data and insufficient in-depth research are the major limitations regarding the medicinal fungus *C. militaris*. Once it was used only by the elite society as a secret of life, but time has changed allowing universal access.

Studies on such novel components of the mushroom *C. militaris* with respect to their efficacy, safety profile, adverse interaction, proper standardization, etc. should be conducted with utmost priority not only by the respective manufacturers but also by the pharmacy, pharmacognosy and medicinal enterprises.

7. Future prospects

Nature is the source of all the raw materials that we need. About 2–3 decades ago, most of the drugs were of herbal origin. A variety of reasons underpin why people like to use natural medicines as it is evident that patients are getting even more distressed after using chemically synthesized drugs, rather than natural means like medicinal mushrooms that can conquer life-claiming diseases, leaving no side effects on human health. To maintain proper growth, the pharmaceutical industries need innovation and access to high output rate on low-cost materials with reasonable safety. The combination of modern chemistry with bio-based starting materials, like, bio-metabolites, offers the scope for revolutionizing mushroom based pharmaceutical industries. In the near future, bio-metabolites (cordycepin, polysaccharides, etc.) extracted from medicinal mushroom like *C. militaris* will have a role that compares with that of oil and gas crackers today.

It is mentionable that the highest cordycepin production was recently obtained in surface liquid culture using *C. militaris* mutant [35]. Therefore, bio-metabolites, such as cordycepin, polysaccharides and alike materials of *C. militaris* will be the key future driving force in the realm of green pharmacology and pharmacognosy.

8. Conclusions

Nowadays, people are willing to wear cotton clothes instead of synthetic/polyester ones, eat vegetables rather than spicy foods and in the same manner, willing to take herbal/natural medicines rather than the synthetic drugs. This is the "back to the pavilion" theory, being more popular day by day. The ancient medicinal fungus *C. militaris*, which has been used as a crude drug for the welfare of mankind in old civilization, is now of a matter of concern due to its unexplored potentials obtained by various modern culture techniques. The anti-cancer agent cordycepin obtained from *C. militaris* have more than 21 clinically approved beneficial effects for human health [3]. Especially, the anti-cancer agent cordycepin from *C. militaris* is expected to play evolutionary roles in the pharmacognosy sector, leading to create a viable base for pharmaceutical industries as some emerging diseases like cancer, SARS, AIDS, swine flu have no proper remedies yet. In this regard, it needs evaluation on modern scientific lines such as accurate phytochemical analysis, biological screening, and gene sequencing, pharmacological investigation and clinical trials.

References

[1] Gu YX, Want ZS, Li SX, Yuan QS. Effects of multiple factors on accumulation of nucleosides and bases in *Corydyceps militaris*. Food Chem 2007;102:1304–9.

[2] Ying J, Mao X, Ma Q, Wen H. Icons of medicinal mushroom from China. Beijing: Science Press Beijing; 1987. p. 151–5. (in Chinese).

[3] Mizuno T. Medicinal effects and utilization of *Cordyceps* (Fr.) Link (Ascomycetes) and *Isaria* Fr. (Mitosporic Fungi) Chinese caterpillar fungi, "Tochukaso" (Review). Intl J Med Mushroom 1999;1:251–61.

[4] Song CH, Jeon YJ, Yang BK, Ra KS, Sung JM. Anti-complementary activity of exo-polymers produced from submerged mycelial cultures of higher fungi with particular reference to *Cordyceps militaris*. J Microbiol Biotechnol 1998;8:536–9.

[5] Nag TB, Wang HX. Pharmacological actions of *Cordyceps*, a prized folk medicine. J Pharm Pharmacol 2005;57:1509–19.

[6] The Mushroom Council Report. February 2006; February 2006 (http://www.mushroomcouncil.com/varieties/varieties.html).

[7] Royse DJ. Forward to the fifth international conference on mushroom biology and mushroom products. Acta Edulis Fungi (Suppl) 2005;12:1–2.

[8] Wasser SP. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl Microbiol Biotechnol 2002;60:258–74.

[9] Lee KH. Research and future trends in the pharmaceutical development of medicinal herbs from Chinese medicine. Public Health Nutr 2000;3 (4A):515–22.

[10] Gogtay NJ, Bhatt HA, Dalvi SS, Kshirsagar NA. The use and safety of non-allopathic Indian medicines. Drug Saf 2002;25(14):1005–19.

[11] Inamdar Nazma, Edalat Shima, Vikram BK, Pawar S. Herbal drugs in milieu of modern drugs. Int J Green Pharm 2008;3–4:2–8.

[12] Burstein HJ, Gelber S, Guadagnoli E, Week JC. Use of alternative medicine by women with early-stage breast cancer. N Engl J Med 1999;340:1733–9.

[13] Strader DB, Bacon BR, Lindsay KL. Use of complementary and alternative medicine in patients with liver disease. Am J Gastroenterol 2002;97:2391–7.

[14] Kassler WJ, Blanc P, Greenblatt R. The use of medicinal herbs by human immunodeficiency virus-infected patients. Arch Intern Med 1999;151:2281–8.

[15] Blanc PD, Trupin L, Earnest G. Alternative therapies among adults with a reported diagnosis of asthma or rhinosinusitis: data from a population-based survey. Chest 2001;120:1461–7.

[16] Rao JK, Mihaliak K, Kroenke K. Use of complementary therapies for arthritis among patients of rheumatologists. Ann Intern Med 1999;131:409–16.

[17] DeSmet PAGM, Keller K, Hansel R, Chandler RF. Adverse effects of herbal drugs, 1. Heidelberg: Springer-Verlag; 1992. p. 1–72.

[18] Winslow LC, Kroll DJ. Herbs as medicines. Arch Intern Med 1998;158:2192–9.

[19] Bateman J, Chapman RD, Simpson D. Possible toxicity of herbal remedies. Scott Med J 1998;43:7–15.

[20] Murphy JM. Preoperative considerations with herbal medicines. Am Org Reg Nurses J 1999;69:173–83.

[21] Mathews HB, Lucier GW, Fisher KD. Medicinal herbs in the United States: research needs. Environ Health Perspect 1999;107(10):773–8.

[22] Cunningham KG, Hutchinson SA, Manson W, Spring FS. Cordycepin, a metabolic product from cultures of *Cordyceps militaris* (Linn.) Link. Part I. Isolation and characterisation. J Chem Soc 1951:2299–3200.

[23] Ling JY, Sun YJ, Zhang PLV, Zhang CK. Measurement of cordycepin and adenosine in stroma of *Cordyceps* sp. by capillary zome electrophoresis (CZE). J Biosci Bioeng 2002;94:371–4.

[24] Ahn YJ, Park SJ, Lee SG, Shin SC, Choi DH. Cordycepin: selective growth inhibitor derived from liquid culture of *Cordyceps militaris* against *Clostridium* spp. J Agric Food Chem 2002;48:2744–8.

[25] Zhao-Long W, Xiao-Xia W, Wei-Ying C. Inhibitory effect of *Cordyceps sinensis* and *Cordyceps militaris* on human glomerular mesangial cell proliferation induced by native LDL. Cell Biochem Funct 2000;18(2):93–7.

[26] Nan JX, Park EJ, Yang BK, Song CH, Ko G, Sohn DH. Antibiotic effect of extracellular biopolymer from submerged mycelial cultures of *Cordyceps militaris* on liver fibrosis induced by bile duct ligation and scission in rats. Arch Pharm Res 2001;24(4):327–32.

[27] Yoo HS, Shin JW, Cho JH, Son CG, Lee YW, Park SY, et al. Effects of *Cordyceps militaris* extract on angiogenesis and tumor growth. Acta Pharmacol Sin 2004;25:657–65.

[28] Choi SB, Park CH, Choi MK, Jun DW, Park S. Improvement of insulin resistance and insulin secretion by water extracts of *Cordyceps militaris*, *Phellinus linteus*, and *Paecilomyces tenuipes* in 90% pancreatectomized rats. Biosci Biotechnol Biochem 2004;68(11):2257–64.

[29] Won SY, Park EH. Anti-inflammatory and related pharmacological activities of cultured mycelia and fruiting bodies of *Cordyceps militaris*. J Ethnopharm 2005;96:555–61.

[30] Park C, Hong SH, Lee JY, Kim GY, Choi BT, Lee YT, et al. Growth inhibition of U937 leukemia cells by aqueous extract of *Cordyceps militaris* through induction of apoptosis. Oncol Rep 2005;13:1211–6.

[31] Masuda M, Urabe E, Sakurai A, Sakakibara M. Production of cordycepin by surface culture using the medicinal mushroom *Cordyceps militaris*. Enzyme Microb Technol 2006;39:641–6.

[32] Masuda M, Urabe E, Honda H, Sakurai A, Sakakibara M. Enhanced production of cordycepin by surface culture using the medicinal mushroom *Cordyceps militaris*. Enzyme Microb Technol 2007;40:1199–205.

[33] Das SK, Masuda M, Hatashita M, Sakurai A, Sakakibara M. A new approach for improving cordycepin productivity in surface liquid culture of *Cordyceps militaris* using high-energy ion beam irradiation. Lett Appl Microbiol 2008;47:534–8.

[34] Das SK, Masuda M, Hatashita M, Sakurai A, Sakakibara M. Optimization of culture medium for cordycepin production using *Cordyceps militaris* mutant obtained by ion beam irradiation. Proc Biochem 2010;45:129–32.

[35] Das SK, Masuda M, Sakurai A, Sakakibara M. Effects of additives on cordycepin production using a *Cordyceps militaris* mutant induced by ion beam irradiation. Afr J Biotechnol 2009;8(13):3041–7.

[36] Cunningham KG, Manson W, Spring FS, Hutchinson SA. Cordycepin, a metabolic product isolated from cultures of *Cordyceps militaris* (Linn.) Link. Nature 1950;166:949–54.

[37] Chatterjee R, Srinivasan KS, Maiti PC. *Cordyceps sinensis* (Berkeley) Saccardo: structure of cordycepic acid. J Am Pharm Assoc 1957;46:114–8.

[38] Kredich NM, Guarino AJ. Homocitrullylaminoadenosine, a nucleoside isolated from *Cordyceps militaris*. J Biol Chem 1961;236:3300–2.

[39] Kaczka EA, Trenner NR, Arison B, Walker RW, Folkers K. Identification of cordycepin, a metabolite of *Cordyceps militaris*, as 3′-deoxyadenosine. Biochem Biophys Res Commun 1964;14:456–7.

[40] Liu JM, Zhong YR, Yang Z, Cui SL, Wang FH. Chemical constituents of *Cordyceps militaris* (L.) Link. Zhongguo Zhong Yao Za Zhi 1989;14:608–9.

[41] Li SP, Li P, Lai CM, Gong YX, Kan KK, Dong TT, et al. Simultaneous determination of ergosterol, nucleosides and their bases from natural and cultured *Cordyceps* by pressurized liquid extraction and high-performance liquid chromatography. J Chromatogr 2004;1036:239–43.

[42] Li SP, Li P, Dong TT, Tsim KW, Wang YT. Anti-oxidation activity of different types of natural *Cordyceps sinensis* and cultured *Cordyceps* mycelia. Phytomedicine 2001;8:207–12.

[43] Li SP, Li P, Dong TT, Tsim KW, Wang YT. Determination of nucleosides in natural *Cordyceps sinensis* and cultured *Cordyceps* mycelia by capillary electrophoresis. Electrophoresis 2001;22:144–50.

[44] Guo C, Zhu J, Zhang C, Zhang L. Determination of adenosine and 3′-deoxyadenosine in *Cordyceps militaris* (L.) Link. By HPLC. Zhongguo Zhong Yao Za Zhi 1998;23:236–7.

[45] Huang LF, Liang YZ, Guo FQ, Zhou ZF, Cheng BM. Simultaneous separation and determination of active components in *Cordyceps sinensis* and *Cordyceps militaris* by LC/ESI-MS. J Pharm Biomed Anal 2003;33:1155–62.

[46] Li YH, Li XL. Determination of ergosterol in *Cordyceps sinensis* and *Cordyceps* black-bone chicken capsules by HPLC. Yao Xue Xue Bao 1991;26:768–71.

[47] Boros C, Hamilton SM, Katz B, Kulanthaivel P. Comparison of balanol from *Vertcillium balanoides* and phiocordin from *Cordyceos ophioglossoides*. J Antibiot (Tokyo) 1994;47:1010–6.

[48] Kneifel H, Konig WA, Loeffler W, Muller R. Ophiocordin, an antifungal antibiotic of *Cordyceps ophioglossoides*. Arch Microbiol 1977;113:121–30.

[49] Isaka M, Kongsaeree P, Thebtaranonth Y. Bioxanthracenes from the insect pathogenic fungus *Cordyceps pseudomilitaris* BCC 1620. II. Structure elucidation. J Antibiot (Tokyo) 2001;54:36–43.

[50] Jaturapat A, Isaka M, Hywel-Jones NL, Lerwerawat Y, Kamchonwong-paisan S, Kirtikara K, et al. Bioxanthracenes from the insect pathogenic fungus *Cordyceps pesudomilitaris* BCC 1620. I. Taxonomy, fermentation, isolation and antimalarial activity. J Antibiot 2001;54:29–35.

[51] Rukachaisirikul V, Chantaruk S, Tansakul C, Saithong S, Chaicharernwimonkoon L, Pakawatchai C, et al. A cyclopeptide from the insect pathogenic fungus *Cordyceps* sp. BCC 1788. J Nat Prod 2006;69:305–7.

[52] Yu RM, Yang W, Song LY, Yan CY, Zhang Z, Zhao Y. Structural characterization and antioxidant activity of a polysaccharide from the fruiting bodies of cultured *Cordycceps militaris*. Charbohydrate Polym 2007;70:430–6.

[53] Kim SW, Hwang HJ, Xu CP, Sung JM, Choi JW, Yun JW. Optimization of submerged culture process for the production of mycelial biomass and

exo-polysaccharides by *Cordyceps militaris* C738. J Appl Microbiol 2003;94:120–6.
[54] Kim SW, Xu CP, Hwang HJ, Choi JW, Kim CW, Yun JW. Production and characterization of exopolysaccharides from an entomopathogenic fungus *Cordyceps militaris* NG3. Biotechnol Progr 2003;19:428–35.
[55] Yu R, Song L, Zhao Y, Bin W, Wang L, Zhang H, et al. Isolation and biological properties of polysaccharide CPS-1 from cultured *Cordyceps militaris*. Fitoterapia 2004;75:465–72.
[56] Yu R, Wang L, Zhang H, Zhou C, Zhao Y. Isolation, purification and identification of polysaccharide from cultured *Cordyceps militaris*. Fitoterapia 2004;75:662–6.
[57] Rukachaisirikul V, Pramjit S, Pakawatchai C, Isaka M, Supothina S. 10-membered macrolides from the insect pathogenic fungus *Cordyceps militaris* BCC 2816. J Nat Prod 2004;67:1953–5.
[58] Huang LF, Guo FQ, Liang YZ, Cheng BM. Determination of adenosine and cordycepin in *Cordyceps sinensis* and *Cordyceps militaris* with HPLC-ESI-MS. Zhongguo Zhong Yao Za Zhi 2004;29:762–4.
[59] Gong YX, Li SP, Li P, Liu JJ, Wang YT. Simultaneous determination of six main nucleosides and bases in natural and cultured *Cordyceps* by capillary electrophoresis. J Chromatogr 2004;A 1055:215–21.
[60] Krasnoff SB, Reategui RF, Wagenaar MM, Gloer JB, Gibson DM. Cicadapeptins I and II: new Aib-containing peptides from the entomopathogenic fungus *Cordyceps heteropoda*. J Nat Prod 2005;68:50–5.
[61] Kawaguchi N, Ohmori T, Takeshita Y, Kawanishi G, Katayama S, Yamada H. Occurrence of Gal beta (1–3) GalNAc-Ser/Thr in the linkage region of polygalactosamine containing fungal glycoprotein from *Cordyceps ophioglossoides*. Biochem Biophys Res Commun 1986;140:350–6.
[62] Watanabe N, Hattori M, Yokoyama E, Isomura S, Ujita M, Hara A. Entomogenous fungi that produce 2,6-pyridine dicarboxylic acid (dipicolinic acid). J Biosci Bioeng 2006;102:365–8.
[63] Jung EC, Kim KD, Bai CH, Kim JC, Kim DK, Kim HH. A mushroom lectin from ascomycete *Cordyceps militaris*. Biochim Biophys Acta Gen Subj 2007;1770:833–8.
[64] Lin WH, Tsai MT, Chen YS, Lai MN, Jeng KCG. Improvement of sperm production in subfertile boars by *Cordyceps militaris* supplement. Am J Chinese Med 2007;35(4):631–41.
[65] Chen C, Luo SS, Li Y, Sun YJ, Zhang CK. Study on antioxidant activity of three *Cordyceps* sp. by chemiluminescence. Shanghai J Trad Chinese Med 2004;38(7):53–5.
[66] Liu J, Yang S, Yang X, Chen Z, Li J. Anticarcinogenic effect and hormonal effect of *Cordycceps militaris*. Zhongguo Yao Za Zhi 1997;22(2):111–3.
[67] Johns DG, Adamson RH. Enhancement of the biological activity of cordycepin (3′-deoxyadenosine) by the adenosine deaminase inhibitor 2′-deoxycoformycin. Biochem Pharmacol 1976;25:1441–4.
[68] Muller WEG, Seihard G, Beyer R, Breter HJ, Maidhof A, Zahn RK. Effects of cordycepin on nucleic acid metabolism in L5178Y cells and on nucleic acid-synthesizing enzymes. Cancer Res 1977;37:3824–33.
[69] Kodama EN, McCaffrey RP, Yusa K, Mitsuya H. Antileukemic activity and mechanism of action of cordycepin against terminal deoxynucleotidyl transferase-positive (TdT^+) leukemic cells. Biochem Pharm 2000;59:273–81.
[70] Penman S, Rosbash M, Penman N. Messenger and heterogenous nuclear RNA in HeLa cells: differential inhibition by cordycepin. Proc Natl Acad Sci USA 1970;67:1878–85.
[71] Shih IL, Tsai KL, Hsieh C. Effects of culture conditions on the mycelial growth and bioactive metabolite production in submerged culture of *Cordyceps militaris*. Biochem Eng J 2007;33:193–201.
[72] Lin YW, Chiang BH. Anti-tumor activity of the fermentation broth of *Cordyceps militaris* cultured in the medium of *Radix astragali*. Proc Biochim 2008;43:244–50.
[73] Sone Y, Okuda R, Wada N. Structures and antitumor activities of the polysaccharides isolated from fruiting body and the growing culture of mycelium of *Ganoderma lucidium*. Agric Biol Chem 1985;49:2641–53.
[74] Mao XB, Zhong JJ. Significant effect of NH_4^+ on cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*. Enzyme Microb Technol 2006;38:343–50.
[75] Park SJ. Growth responses of intestinal microorganisms to tochukaso, mushroom and tropical plant, and cordycepin from *Cordycceps militaris*. M. S. Thesis. Seoul National University, Suwon, Republic of Korea, 1996.
[76] Ahn YJ, Park SJ, Lee SG, Shin SC, Choi DH. Cordycepin: selective growth inhibitor derived from liquid culture of *Cordycceps militaris* against *Clostridium* spp. J Agric Food Chem 2000;48:2744–8.
[77] De Julian-Ortiz JV, Galvez J, Munoz-Collado C, Garcia-Domenech R, Gimeno-Cardona C. Virtual combinatorial syntheses and computational screening of new potential anti-herpes compounds. J Med Chem 1999;17:2208–3314.
[78] Mueller WEG, Weiler BE, Charubala R, Pfleiderer W, Leserman L, Sobol RW, et al. Cordycepin analogues of 2′5′-oligoadenylate inhibit human immunodeficiency virus infection via inhibition of reverse transcriptase. Biochemistry 1991;30:2027–33.
[79] Trigg P, Gutteridge WE, Williamson J. The effect of cordycepin on malarial parasites. Trans R Soc Trop Med 1971;65:514–20.
[80] Kim JR, Yeon SH, Kim HS, Ahn YJ. Larvicidal activity against *Plutella xylostella* of cordycpin from the fruiting body of *Cordycceps militaris*. Pest Manage Sci 2002;58:713–7.
[81] Shen Q, Chen S. Effect of *Cordycceps militaris* on the damage of rats induced by n-hexane. Zhong Yao Cai 2001;24:112–6.
[82] Sugar AM, McCaffrey RP. Antifungal activity of 3′-deoxyadenosine (cordycepin). Antimicrob Agent Chemother 1998;42(6):1424–7.
[83] Jung K, Kim IH, Han D. Effect of medicinal plant extracts on forced swimming capacity in mice. J Ethmopharmacol 2004;93:75–81.
[84] Ribeizo JA. Purinergic inhibition of neurotransmitter release in the central nervous system. Pharm Toxicol 1995;77(5):299–305.
[85] Wu ZL, Wang XX, Cheng WY. Inhibitory effect of *Cordycceps sinensis* and *Cordycceps militaris* on human glomerular mesangial cell proliferation induced by native LDL. Cell Biochem Funct 2000;18:93–7.
[86] Hui MY, Wang BS, Shiow CH, Duh PD. Comparison of protective effects between cultured *Cordyceps militaris* and natural *Cordyceps sinensis* against oxidative damage. J Agric Food Chem 2006;54:3132–8.
[87] Aman S, Anderson DJ, Connolly TJ, Crittall AJ, Ji G. From adenosine to 3′-deoxyadenosine: development and scale up. Org Process Res Dev 2000;4:601–5.
[88] Hansske F, Robins MJ. Regiospecific and stereoselective conversion of ribonucleosides to 3′-deoxynucleosides. A high yield three-stage synthesis of cordycepin from adenosine. Tetrahedron Lett 1985;26:4295–8.
[89] Jiang XL, Sun Y. The determination of active components in various *Cordyceps militaris* strains. Acta Edulia Fungi 1999;6(1):47–50.
[90] Tong YK, Kuang T, Wu YX, Zhang QY, Ren J. Comparation of components of *Cordyceps* mycelium and natural *Cordyceps sinensis*. Shi Pin Yan Jiu Yu Kai Fa 1997;18(4):40–2.