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In vitro bioactive potential and chemical analysis of the n-hexane extract of the medicinal mushroom, *Cordyceps militaris*

Ali El-Hagrassi[1], Ghoson Daba[2], Waill Elkateeb[2]*, Eman Ahmed[2], Asmaa Negm El-Dein[2], Walid Fayad[3], Mohamed Shaheen[4], Riham Shehata[4], May El-Manawaty[3] and Ting-Chi Wen[5]*

[1]Phytochemistry and Plant Systematic Department, Pharmaceutical industries Division, National Research Centre, Dokki, Giza, 12622, Egypt.
[2]Chemistry of Natural and Microbial Products Department, Pharmaceutical Industries Division, National Research Centre, Dokki, Giza, 12622, Egypt.
[3]Drug Bioassay-Cell Culture Laboratory, Pharmacognosy Department, National Research Centre, Dokki, Giza, 12622, Egypt.
[4]Environmental Virology Laboratory, Water Pollution Research Department, Environmental research Division, National Research Centre, Dokki, Cairo, Egypt.
[5]Engineering Research Center of Southwest Bio-Pharmaceutical Resources, Ministry of Education, Guizhou University, Guiyang 550025, Guizhou Province, China.
Email: waillahmed@yahoo.com; tingchiwen@yahoo.com

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ABSTRACT

Aims: *Cordyceps militaris* is a medicinal mushroom from Ascomycota. The aims of this study were to explore and identify the chemical compounds extracted in the non-polar fraction of the mushroom and to examine the biological potential of this extract.
Methodology and results: The n-hexane extract metabolites were identified using gas chromatography-mass spectrometry (GC-MS) and results revealed the presence of 37 compounds delivered from different chemical classes and were mainly comprised of fatty acids and their esters (72%), carboxylic acids and their esters (10.39%), and a sulphur compound (7.1%). The n-hexane extract recorded a promising antioxidant effect (80.9±1.5%) at 80 mg/mL total extract; potent cholesterol reduction activity (100%) was obtained after 96 h incubation by the total metabolites (4%). The cytotoxicity of the compounds revealed 50% cytotoxicity concentration (CC_{50}) > 1 mg/mL and anti-rotavirus SA-11 effect where inhibition of virus attachment and penetration into infected cells was recorded at 50% effective concentration (IC_{50}) of 300±0.2 µg/mL.
Conclusion, significance and impact of study: This study confirmed the impact of the fatty acids produced by *C. militaris* as bioactive metabolites**.**

Keywords: Medicinal mushroom, *Cordyceps militaris*, antiviral, GC-MS analysis, fatty acids

INTRODUCTION

Mushrooms are a natural mine of biologically active compounds, which are responsible for their therapeutic reputation and were used in old East Asia conventional medicines. One of the most famous medicinal mushrooms is the insect mushroom, *C. militaris* (the orange caterpillar fungus), which is an Ascomycetous fungus that parasites on caterpillars (Smiderle *et al*., 2014), though it was previously reported to grow also on some grains such as germinated soybeans (Ohta *et al*., 2007), rice grain (Hong *et al*., 2010) and spent brewery grains (Gregori, 2014).

Cordyceps militaris gains a superior attention over other *Cordyceps* species since it can be cultivated artificially (Yang *et al*., 2012), also due to the continuously updated list of biologically active compounds extracted from its fruiting bodies. Cordycepin (3-deoxyadenosine) is one of the most important biologically active compounds extracted from *C. militaris.* Cordycepin exhibits antitumor, antimetastatic, and immunomodulatory activities (Cunningham *et al*., 1951; Jeong *et al*., 2013; Wang *et al*., 2017), inhibit human immunodeficiency virus infection (Mueller *et al*., 1991), antimicrobial (Ahn *et al*., 2000) and insecticidal activities (Kim *et al*., 2002). Additionally cordycepin has hypolipidaemic (Gao *et al*., 2011), antiasthmatic effects (Tianzhu *et al*., 2015), prevents hyperglycemia (Ma *et al*., 2015) and prevents hyper-

***Corresponding author**

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glycemia hyperuricemia (Yong *et al*., 2018).

Polysaccharides extracted from *C. militaris* have a wide range of biological activities. They inhibited cancer growth in S180 mice and reduced significantly toxicity side effect of chemotherapy (Zhong *et al*., 2008). They also showed antioxidant activity *in vitro* (Wang *et al*., 2012). Moreover, *C. militaris* originated polysaccharides have an immunostimulatory effect and could reduce the *in vivo* melanoma growth in experimental mouse (Lee and Hong, 2011). On the other hand, cordymin extracted from *C. militaris* has over 21 approved medical applications in the field of human health especially as antifungal, anti-proliferative and anti-cancer (Mizuno, 1999; Wong *et al*., 2011).

Furthermore, *C. militaris* extracts contain other biometabolites such as cordycepic acid (D-mannitol), flavonoids, glucans, macrolides, and sterols (ergosterol) (Rukachaisirikul *et al*., 2004; Wong *et al*., 2011; Nallathamby *et al*., 2015; Sun *et al*., 2017). These compounds deliver various biological activities including antiviral, anti-inflammatory, antibacterial, antitumor, anti-proliferative, anti-metastatic, anti-angiogenetic, hypoglycaemic, anti-fibrotic and immunomodulatory activities (Ng and Wang, 2005; Won and Park, 2005; Paterson and Russel, 2008; Yang *et al*., 2012; Hung and Lee, 2017). Moreover, some *C. militaris* extracts have a role in improving insulin secretion as well as insulin resistance (Choi *et al*., 2004).

However, available information about the medicinal properties of *C. militaris* is mainly focusing on those related to its polysaccharides and cordycepin extracts. Therefore, in this study, the antioxidant, hypoglycemic, anti-rota virus, as well as cholesterol reducing properties of the n-hexane extract of *C. militaris* were investigated. Moreover, GC-MS analysis was conducted in order to elucidate metabolites existing in this *C. militaris* n-hexane extract.

MATERIALS AND METHODS

Sample and extraction

Cordyceps militaris was brought from Guizhou, Tianqi, Wildlife, sources, conservation R&D Centre in Guiyang city, Guizhou province, China. Two hundred and fifty grams of *C. militaris* fruiting bodies were washed with distilled water, air dried before cut into small pieces and placed in an Erlenmeyer flask containing n-hexane at room temperature and kept for 48 h prior to filtering. The resulting filtered extract was concentrated at 37 °C using a rotary evaporator. Obtained extract was stored at 4 °C in a clean closed container until further use.

GC-MS analysis

The analysis of the n-hexane extract was performed using a GC-MS instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center, Egypt with the following specifications. Instrument: TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-WAX MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 60 °C for 1 min; rising at 3.0 °C /min to 240 °C and held for 1 min. The injector and detector were held at 240 °C. Diluted samples (1:10 hexane, v/v) of 0.2 µL of the mixtures were always injected automatically in splitless mode. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library). The quantification of the components was based on the metabolites as detected by the mass spectrometer. Identification of the constituents was carried out by comparison of their retention times and fragmentation pattern of mass with those of published data assay (Adams, 1995) and with those of the Wiley 9 and NIST08 mass spectra library.

Antioxidant scavenging activity

DPPH (1-diphenyl-2-picrylhydrazyl) scavenging activity was measured as described by Lee *et al.* (2010). Briefly, 500 µL of ethanolic DPPH solution (0.4 mmol) was vigorously mixed vigorously with 500 µL of *C. militaris* n-hexane extract (80 mg/mL) or water (as a control) and incubated at 37 °C in the dark for 1 h. The absorbance of the mixture was measured spectrophotometrically at 517 nm. The percentage of scavenging activity was calculated as $[1-(A_s - A_b)/A_c] \times 100$, whereas A_b, A_c and A_s is the absorbance of the blank (ethanol and sample), the control (DPPH and deionised water) and the sample (DPPH and sample), respectively. Ascorbic acid at the concentration of 0.1% was used as positive control.

Cell culture

HCT116 colon carcinoma human tumor cell lines were cultured in 95% humidity, 5% CO_2 and 37 °C. The cell line was maintained in McCoy's 5A medium supplemented with 10% fetal bovine serum.

Cytotoxicity on HCT116 cell line monolayers

The acid phosphatase assay was used to assess cytotoxicity according to the method described by Yang *et al.* (2012). A number of cells (1000) were seeded per well in 96 well microtiter plates, left to attach overnight, and then treated with samples for 3 days. For one plate, a substrate solution was prepared where 20 mg tablet of pNPP (Sigma; cat. no. N2765) was dissolved in 10 mL buffer solution (0.1 M sodium acetate, 0.1% triton X-100, pH 5.0). Cell monolayers were washed with 250 µL PBS. pNPP substrate solution (100 µL) was added per well, then plates were incubated for 4 h at 37 °C. Sodium

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hydroxide (1N) stop solution was used by adding 10 µL per well. Absorbance was measured directly at wavelength 405 nm. All samples were tested in triplicates, and 0.5% DMSO was used as negative control and 50 µM cisplatin was used as the positive control. Samples were tested at serial dilutions with final concentration of 100, 50, 25 and 12.5 µg/mL. Percent cytotoxicity was calculated by the formula:

Cytotoxicity = [1-(D/S)] × 100, where D and S denote the optical density of drug and solvent treated wells, respectively.

In vitro cholesterol reduction by *C. militaris* n-hexane extract

Different concentrations of *C. militaris* n-hexane extract (0.5-4%) were prepared. Soluble cholesterol (1 mL) was added to 4 mL of each concentration and incubated at room temperature for 24, 48, 72 and 96 h. To determine the residual amount of cholesterol, the cholesterol assay kit (Biodiagnostic, Egypt) was used and the percentage of cholesterol reducing activity was assessed. The control was prepared by adding distilled water (4 mL) to the soluble cholesterol (1 mL). The percentage of cholesterol reducing activity was calculated as describes previously (Pan *et al.*, 2005) as follows:

Cholesterol reducing activity (%) = $[(A_0 - A)/A_0] \times 100$,
where A_0: absorbance of the control (500 nm); A: absorbance of the sample (500 nm). Tests were carried out in triplicate.

Antidiabetic activity (α-amylase inhibitory activity)

The α-amylase inhibition activity of the n-hexane extract of *C. militaris* was investigated as described by Xiao *et al.* (2006). The enzyme α-amylase (4 U/mL) was prepared in phosphate buffer solution (pH 6.8). Different concentrations of *C. militaris* extract (0.6-220) ppm were prepared and 15 µL of each concentration was mixed with 60 µL of α- amylase (4 U/mL) and incubated in a 96 well microtiter plate for 15 min at 37 °C. Soluble starch solution (0.2%) was dissolved by heating in a microwave, filtered and 60 µL of the prepared solution was added and incubated at 37 °C for 10 min. The reaction was stopped by the addition of 30 µL of 1M HCl and 150 µL KI/I_2 aqueous solution. The α-amylase activity was determined spectrophotometrically at 595 nm by measuring the quantity of the released blue color. In the negative control 15 µL of buffer solution was used instead of the sample. Acarbose was used as a positive control. The calculation and analysis of data were done using GraphPad Prism programme.

Cell lines and virus titration

Rhesus monkey kidney cell line (MA 104) were used for culturing of simian rotavirus SA-11 strains. MA 104 cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM). The media were provided with 10% heat inactivated fetal bovine serum (FBS), 100 µg/mL streptomycin and 100 units/mL penicillin, 1% HEPES (4-2-hydroxyethyl-1-piperazineethanesulfonic acid). The cell culture was incubated under humidified 5% CO_2 atmosphere in CO_2 incubator. The medium used for both cytotoxicity and antiviral assays contained only 2% of fetal bovine serum. RV SA-11 for antiviral experiments was activated by 10 mg/mL trypsin for 30 min at 37 °C. RV SA-11 stock was titrated using MA 104 in 96 well microtiter plates as described previously by Shaheen *et al*. (2015). The viral titers were calculated as $TCID_{50}$/0.1 mL (50% tissue culture infectious doses/0.1 mL) according to Spearman Kärber formula (Finney, 1952). RV SA-11 stock was kept in small aliquots at –80 °C until use.

Cytotoxicity assay

Different concentrations of *C. militaris* n-hexane extract (7.8, 15.6, 31.25, 62.5, 125, 250, 500, and 1000) µg/mL were prepared in DMEM (containing 2% FBS and 2% antibiotics). The cytotoxic activity of the tested extract was examined onto MA 104 by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay (Nabil *et al*., 2012). Briefly, the cell lines (5×10^3 and 5×10^4 cells/well) were seeded in 96 well microtiter plates. After 24 h in 5% CO_2 incubator at 37 °C, the cell monolayers were treated with various concentrations of the extract (each dilution in triplicate). Cell control was used without sample treatment. The treated or non-treated cells were incubated for two days at 37 °C in a 5% CO_2 incubator with checking the cell morphology under inverted microscope daily. After the previous incubation period, the culture medium was discarded and replaced by 100 µL of MTT solution (5 mg/mL) for 4 h at 37 °C in CO_2 incubator. After that, the MTT solution was removed and replaced by 50 µL DMSO/ well. After 30 min at 37 °C, the optical densities (OD) were measured using ELISA reader at 540 nm. The percentage of cytotoxic effects was calculated as [(C - T / C) × 100], where C refers to the average of three optical densities of cell control and treated cells, respectively.

Antiviral activity

MA 104 at a concentration of 5×10^4 cells/well were cultured for 24 h in a CO_2 incubator at 37 °C in the 96 well microtiter plates. After removing the culture media, three non-toxic concentrations of the extract were tested against viral infections. Aliquot of 50 µL of 10^6 $TCID_{50}$ virus suspensions was incubated with 50 µL of culture media (with or without the test compound) in a humidified 5% CO_2 atmosphere for 1 h at 37 °C then the mixed solution was added to cell monolayers. After 1 h in a CO_2 incubator, the mixed solution was removed. The cell lines were washed two times with culture medium then 200 µL infectious medium (FBS free DMEM containing 2 µL of trypsin) was added to cells. Virus controls, containing the virus suspension, and cell controls, containing culture

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medium, were included in the assay. All plates were incubated for 3 days at 37 °C in a CO_2 incubator and the cytopathic effect of the virus was monitored daily then measured by the MTT as described above. The percentage of protection was calculated as [(T-V)/(C-V) × 100], where T, V and C are the absorbance readings of the extract with virus, virus control, and cell control, respectively. Therapeutic index (TI) of the tested extracts was calculated as the ratio of CC_{50} over IC_{50}.

RESULTS AND DISCUSSION

GC- MS analysis

The results of GC-MS analysis on n-hexane extract of *C. militaris* indicated the presence of 37 compounds which were identified and classified into different classes (Table 1 and Figure 1). The major constituent was the fatty acids and their esters class where 16 compounds were identified and represented 72% of the total compounds. Of all compounds only five were classified as carboxylic acids and their esters (10.39%).The major peak areas was 30.2% for 9,12-octadecadienoic acid, methyl ester, (E,E)-(Linolelaidic acid, methyl ester).The previous results were obtained by the use of computer searches on NIST Ver.2.1 MS data library and by comparing the spectrum obtained through GC-MS of the compounds present in the plant sample. The individual compounds from n-hexane extract were identified based on direct comparison of the retention times and their mass spectra with the spectra of known compounds stored in the spectral database, NIST (version year 2005).

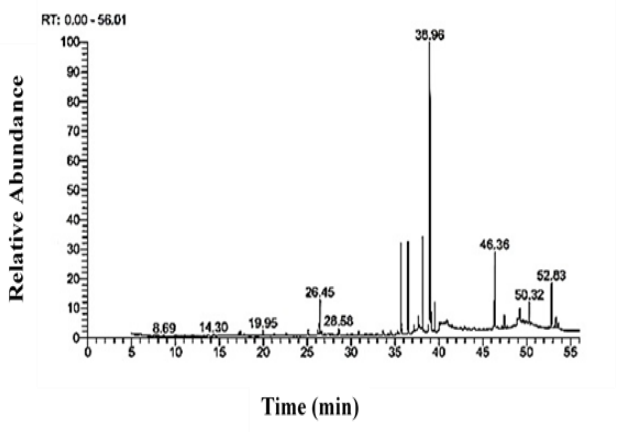

Figure 1: Total ion chromatogram of *C. militaris* n-hexane extract.

The majority of the compounds in the chemical profile were related to fatty acids, carboxylic acids and their esters which were known for their biological activities (antioxidant, anticancer and anti-cholesterol). Some of the identified compounds didn't possess any biological activities, while the others were reported to have potent activities.

The aromatic alcohols compound Phenol, 2,4-bis (1,1-dimethylethyl) was reported as an antioxidant compound. Heptadecanoic acid, methyl ester (1.27%) and 9-octadecenoic acid (Z)-, methyl ester (Methyl oleate) (2.35%) were known as anticholesterol effect. Additionally, linoleic acid ethyl ester (Ethyl linoleate) has a potent cytotoxic effect on cancer cells.

Antioxidant activity of the *C. militaris* n-hexane extract

Scavenging of DPPH free radical assay was used for antioxidant assay and it was attributed to the hydrogen donating abilities of antioxidants. The antioxidant potential of *C. militaris* metabolites was evaluated as shown in Figure 2. N-hexane extract exhibited a potent activity (80.9±1.5%). Previous reports confirmed the anti- DPPH oxidation of aqueous and ethanol extracts of *C. militaris*, (Yuxiang *et al*., 2006). Based on the reported information we could suggest that this activity was related to the presence of the bioactive aromatic alcohols compounds; Phenol, 2,4-bis (1,1-dimethylethyl) (Ajayi *et al.*, 2011).

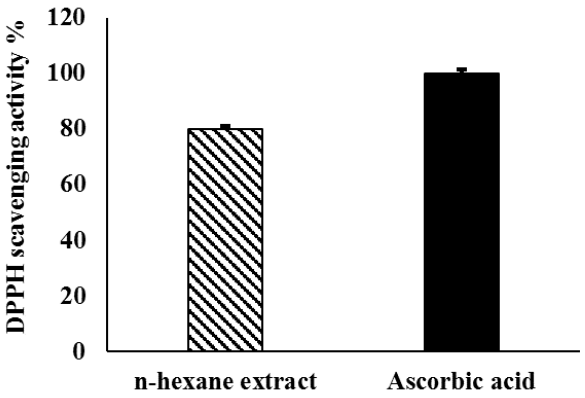

Figure 2: Antioxidant activity of ascorbic acid (positive control), and *C. militaris* n-hexane extract at 80 mg/mL. Error bars represent the standard deviation of three independent experiments.

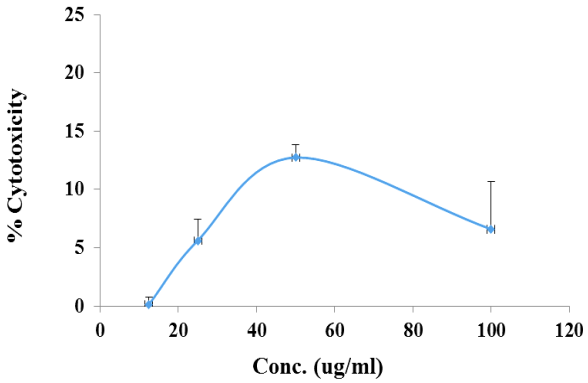

Figure 3: Cytotoxicity of HCT116 cell line monolayers by *C. militaris* n-hexane extract. Error bars represent the standard deviation of three independent experiments.

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Hypocholesterolemic potential of *C. militaris* metabolites

The results shown in Table 2 revealed a gradual increase in cholesterol reduction activity with the increasing concentration of the extract as well as the increase in the incubation time of each concentration. The highest reducing activity of *C. militaris* was achieved after 96 h of incubation with the highest concentration (4%) of the n-hexane extract. GC-MS analysis revealed the presence of Heptadecanoic acid, methyl ester, 9-octadecenoic acid (Z)-, methyl ester (Methyl oleate). These results suggest involvement of fatty acids compounds in cholesterol reduction as described previously (Hema *et al.*, 2011).

Table 1: GC-MS analysis of *C. militaris* n-hexane extract.

No.	Name of the compounds	Molecular formula	Retention time (min)	Molecular weight	Peak area (%)
i)	Hydrocarbons				
1	Pentadecane	$C15H_{32}$	26.16	212	0.65
2	Hexadecane	$C_{16}H_{34}$	28.58	226	0.60
3	Docosane	$C_{22}H_{46}$	30.85	310	0.36
ii)	Fatty alcohol				
4	1,16-Cyclocorynan-16-methanol,19,20-didehydro-17-hydroxy-10-methoxy-, (19E)-	$C_{21}H_{26}N_2O_3$	42.92	354	0.45
iii)	Carboxylic acids and their esters				
5	Heptanoic acid, trimethylsilyl ester	$C_{10}H_{22}O_2Si$	17.20	202	0.35
6	Octanoic acid, trimethylsilyl ester	$C_{11}H_{24}O_2Si$	19.96	216	0.52
7	Decanoic acid, trimethylsilyl ester	$C_{13}H_{28}O_2Si$	25.11	244	0.62
8	Methyl1,3-dihydro-2H-isobenzofuran-4-carboxylate	$C_{10}H_{10}O_3$	36.50	187	8.45
9	Mono(2'-ethylhexyl) hexanedioate	$C_{14}H_{26}O_4$	38.02	258	0.45
iv)	Phenolic ester				
10	1,2-Benzenedicarboxylic acid, butyl2-ethylhexyl ester	$C_{20}H_{30}O_4$	34.55	334	0.44
v)	Halogenated				
11	Androstane, 17,18-diiodo-, (5à,17á)-	$C_{19}H_{30}I_2$	37.75	512	0.98
vi)	Sulphur Compounds				
12	Bis(6-methylthieno[3',2':8,9]bicyclo[4.3.0]non-1(2)-en-3-ylidene)	$C_{24}H_{24}S_2$	52.83	376	7.10
vii)	Ketones				
13	1,4-Hexadien-3-one,5-methyl-1-[2,6,6-trimethyl-2,4-cyclohexadien-1-yl]-	$C_{16}H_{22}O$	47.48	230	1.38
14	3-Methyl-3,9,13-triazapentacyclo[8.7.0.1(2,6).0(5,7).0(14,19)]nonadeca-1,4,14,15,17-pentaen-8,10-dione	$C_{17}H_{15}N_3O_2$	49.60	293	0.36
15	3á-Acetoxy-5à-pregnan-18,20-dione	$C_{23}H_{34}O_4$	52.56	374	0.35
viii)	Triterpene				
16	Supraene	$C_{30}H_{50}$	50.32	410	2.33
17	Tetracosapentaene, 2,6,10,15,19,23-hexamethyl (Dihydrosqualene)	$C_{30}H_{52}$	49.76	412	0.45
ix)	Steroids				
18	2,3-Secocholestan-2,3-dioic acid	$C_{27}H_{46}O_4$	53.60	434	0.82
iix)	Fatty acids and their esters				
19	Murrangatin isovalerate	$C_{20}H_{24}O_6$	26.45	360	3.20
20	Tetradecanoic acid, 12-methyl-, methyl ester	$C_{16}H_{32}O_2$	33.66	256	0.56
21	Pentadecanoic acid,14-methyl-, methyl ester	$C_{17}H_{34}O_2$	35.70	280	8.17
22	9,12-Octadecadienoi acid (Z,Z)) (Linoleic acid)	$C_{18}H_{32}O_2$	37.18	280	0.81
23	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	37.64	284	1.27
24	Hexanoic acid, 2-ethyl-, anhydride	$C_{16}H_{30}O_3$	38.15	270	8.05
25	9,12-Octadecadienoic acid, methyl ester, (E,E)- (Linolelaidic acid, methyl ester)	$C_{19}H_{34}O_2$	38.96	294	30.2
26	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	39.05	296	2.35

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	(Methyl oleate)				
27	Octadecanoic acid, methyl ester (Methyl stearate)	$C_{19}H_{38}O_2$	39.51	298	4.62
28	Linoleic acid ethyl ester (Ethyl linoleate)	$C_{20}H_{36}O_2$	40.13	308	2.09
29	9-Octadecenoic acid (Z), 2-hydroxy-1,3-propanediyl ester	$C_{39}H_{72}O_5$	40.89	620	0.41
30	cis-10-Nonadecenoic acid	$C_{19}H_{36}O_2$	41.52	296	0.40
31	(2E,4S,6S,8S)-2,4,6,8-Tetramethyl-2-undecenoic acid	$C_{15}H_{28}O_2$	46.36	240	7.03
32	9,12-Octadecadienoic acid (Z,Z)-,1,2,3-propanetriyl e ster (Trilinolein)	$C_{57}H_{98}O_6$	48.47	878	0.38
33	Linoleic acid ethyl ester (Ethyl linoleate)	$C_{20}H_{36}O_2$	48.96	308	0.96
34	Ethyl 3,7,12-trihydroxycholan-24-oate (Ethyl iso-allocholate)	$C_{26}H_{44}O_5$	49.21	436	1.46
Iiix) Dicarboxylic ester					
35	1,2-Benzenedicarboxylic acid, dinonyl ester (Phthalic acid, dinonyl ester)	$C_{26}H_{42}O_4$	35.32	418	0.45
Vx) Aromatic alcohols					
36	Phenol, 2,4-bis (1,1-dimethylethyl)	$C_{14}H_{22}O$	26.62	206	0.38
Vix) Cyclo ether					
37	D- Erythro-pentopyranose , 2-deoxy, tris-O-trimethylsilyl)-	$C_{14}H_{34}O_4Si_3$	17.41	350	0.49

Table 2: *In vitro* reducing activity (CRA) of *C. militaris* n-hexane extract.

Concentration of the n-hexane extract (%)	*Cordyceps militaris* CRA (%)			
	Incubation time (h)			
	24	48	72	96
0.5	70.7±1.80	72.8±1.10	75.7±1.42	91.4±1.05
1.0	71.4±1.20	74.0±0.95	77.9±2.05	91.4±2.32
2.0	72.9±0.60	76.5±1.20	80.7±1.30	92.1±1.90
3.0	76.4±0.90	81.7±1.00	87.1±0.60	97.9±0.80
4.0	77.9±0.40	83.1±0.90	89.3±0.90	100.0±0.00

Each value represents the mean of three replicates (Mean ±SD).

Cytotoxicity of *C. militaris* n-hexane extract on HCT116 cell line monolayers

The results of cytotoxicity which were given in Figure 3 recorded a weak inhibition of mushroom extract to HCT116 cell line at all concentrations. The maximum effect was shown at 50 µg/mL where 12.7% inhibition of cells growth was achieved. The fatty acids: 9-Octadecenoic acid (Z)-, methyl ester (Methyl oleate) (Hema *et al.*, 2011) and Linoleic acid ethyl ester (Ethyl linoleate) (Jargalsaikhan *et al.*, 2013) were found to have a cytotoxic effect on cancer cells.

Hypoglycemic effect of the n-hexane extract of *C. militaris*

The results in Figure 4 confirmed a weak inhibition of n-hexane metabolites to α-amylase (18.8±0.78%) at 55 ppm compared with acarbose (100%) which led to an increase in the levels of sugar in the medium.

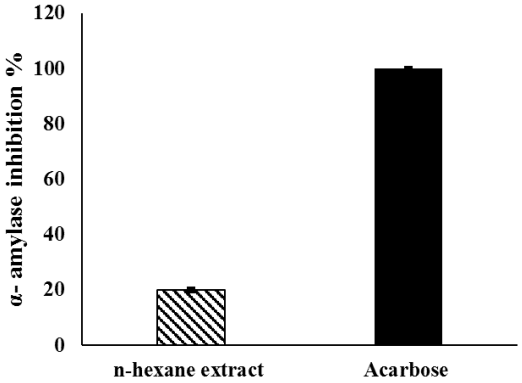

Figure 4: The α-amylase inhibition (%) of n-hexane extract of *C. militaris*, and acarbose as positive control. Error bars represent the standard deviation of three independent experiments.

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Table 3: Results of cytotoxicity and antiviral activities of the tested extract on MA104 cells determined by MTT method.

Antiviral activity	CC_{50} (µg/mL)[a]	IC_{50} (µg/mL)[b]	TI[c]
C. militaris n-hexane extract	6000±0.6	300±0.2	20

[a] 50% cytotoxic concentration
[b] 50% inhibitory concentration
[c] Therapeutic index (CC_{50} / IC_{50})

Cytotoxicity and anti- RV SA-11 effect

N-hexane extract of *C. militaris* was investigated for its cytotoxic effect on MA 104 cells using MTT colorimetric assay. The $CC_{50,}$ IC_{50} and therapeutic index values of the tested extract against MA 104 cells was evaluated as presented in Table 3.

Viral replication cycle includes various steps such as attachment, penetration, replication of viral proteins and genetic materials, assembly and viral escaping from infected cells. These steps can be used as targets of anti-rotavirus SA-11 agents (Estes and Kapikian, 2007). In this study, we determined the effect of tested *C. militaris* n-hexane extract on the attachment step and penetration steps. As shown in Table 3. *Cordyceps militaris* showed CC_{50} higher than 1 mg/mL and the obtained IC_{50} was 300±0.2 µg/mL, with therapeutic index 20. These results suggested that this extract has an antiviral activity.

CONCLUSION

In the current study, the n-hexane extract of the caterpillar mushroom, *C. militaris*, was evaluated for its bioactive compounds. The extract exhibited promising cholesterol-reducing capability, antioxidant potential and anti- RV SA-11 activities. The chemical profile of the compounds revealed detection of 37 compounds belonging to different chemical classes. Fatty acids comprised about 72% of total metabolites. The presence of the bioactive aromatic alcohols compounds; Phenol, 2,4-bis (1,1-dimethylethyl) could be suggested as the reason for the DPPH scavenging activity of the extract. On the other hand, detection of fatty acids such as heptadecanoic acid, methyl ester, 9-octadecenoic acid (Z), and methyl ester (Methyl oleate) in the n-hexane extract of *C. militaris* could be involved in cholesterol reduction ability as described previously. The present study highlighted the importance of the medicinal mushroom, *C. militaris*, as a promising source of valuable therapeutic compounds which could be used as alternatives to synthetic drugs.

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