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Therapeutic potentials of n-hexane extracts of the three medicinal mushrooms regarding their anti-colon cancer, antioxidant, and hypocholesterolemic capabilities

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Abstract. *Daba GM, Elkhateeb WA, El-Dien AN, Ahmed EF, El Hagrassi AM Fayad W, Wen TC. 2020. Therapeutic potentials of n-hexane extracts of the three medicinal mushrooms regarding their anti-colon cancer, antioxidant, and hypocholesterolemic capabilities. Biodiversitas 21: 2437-2445.* Evaluating *in vitro* biological activities of the medicinal mushrooms *Hericium erinaceus, Metacordyceps neogunnii,* and *Dictyophora indusiata* n-hexane extracts revealed their capabilities as promising therapeutic sources. By assessing their DPPH radical scavenging activities, *D. indusiata* extract exhibited the highest antioxidant activity (87.8±1.2%) followed by *H. erinaceus*, then *M. neogunnii* 84.9±1.6%, and 77.3±1.3%, respectively. On the other hand, *M. neogunnii* extract exerted promising anti-colon cancer (68.6±3.6% cytotoxicity) against HCT116 human colon cancer cell lines at concentration 100 µg/mL, whereas *H. erinaceus* and *D. indusiata* extracts exhibited weaker cytotoxic effects (18.3±1.7%, and 19.3±3.2%, respectively) using the same concentration. Investigating cholesterol reduction activities (CRA%) of the extracts revealed that activity depends on both extract concentration and incubation period. After 96 h of incubation at room temperature, all extracts exerted potent *in vitro* hypocholesterolemic activities (100±0% reduction in cholesterol concentration). Performing GC-Ms analyses on the extracts revealed presence of 22 compounds in *D. indusiata* extract, while 29 compounds were detected in *M. neogunnii* extract, and 33 in *H. erinaceus* extract. Most of compounds were esters of fatty acids. Results in this study encourage using these mushrooms as functional foods, and highlight the need for conducting further *in vivo* studies to support their use in pharmaceutical industry.

Keywords: Anti-colon cancer; antioxidant; *Dictyophora indusiata*; *Hericium erinaceus*, hypocholesterolemic; *Metacordyceps neogunnii*

INTRODUCTION

Reducing cholesterol levels is one of the key factors controlling cardiovascular diseases (CVD) through decreasing risk of developing some heart diseases (Jeong et al. 2018). Reports of WHO have specified CVD as the reason for mortalities of about 18 million people annually which represents 31% of total number of mortalities all over the world (WHO fact sheet, 2017). Currently used HMG-CoA reductase inhibitors (statins) prescribed for cholesterol-lowering purpose have some serious side effects such as statin-associated muscle symptoms, pancreatic and hepatic dysfunction, diabetes mellitus, neuropathy, sexual dysfunction, cognitive loss, and central nervous system complaints (Golomb and Evans, 2008; Thompson et al. 2016). These serious adverse effects made researchers investigate for different agents having cholesterol-reducing capabilities. On the other hand, colon cancer is one of the major cancers responsible for elevated number of mortalities worldwide. Colorectal cancer alone is the cause of mortalities approaching 1 million people in 2018 (WHO fact sheet, 2018). Hence, all efforts are focusing nowadays on screening for sources rich in compounds displaying anticancer properties.

Mushrooms are group of macrofungi belong to divisions Ascomycota and Basidiomycota. Consumption of mushrooms as food or for their medicinal properties is known especially in Asian countries from millennia (Chang and Miles, 1989; Sullivan et al. 2006; Wang et al. 2018; Elkhateeb et al. 2019a). *Metacordyceps neogunnii,* or as known previously, *Paecilomyces gunnii* (its asexual morph, Liang 1985), is an entomogenous fungus used in Asian countries as a medicinal mushroom (Wen et al. 2017). For over 30 years, this mushroom has been wrongly regarded as *Cordyceps gunnii* but currently, it has been proven that it differs morphologically from similar species since it has longer asci and wider ascospores. Furthermore, multigene analysis and sequence data had confirmed the uniqueness of this species (Wen et al. 2017). Generally, Chinese *Cordyceps* species are ranked among the expensive natural alternatives due to their medicinal capabilities (Bashir et al. 2017). One kilogram of this mushroom costs about 2000$, and it is recognized as an alternative for the famous mushroom *Ophiocordyceps sinensis.* However, precise studies discussing the biological activities of

Metacordyceps neogunnii extracts are remarkably rare. One of the important edible mushrooms is *Hericium erinaceus* which is a well-studied medicinal mushroom known for centuries in Chinese traditional medicine as well as South American and European literature (Thongbai et al. 2015). Many biological activities have been reported for the fruiting bodies extracts of *H. erinaceus* such as having haemagglutinating, anti-aging, immunomodulatory, hypoglycemic, antimicrobial, antiproliferative, and antitumor activities (Mizuno et al. 1992; Gong et al. 2004; Yim et al. 2007; Li et al. 2010; Zhang et al. 2012; Thongbai et al. 2015; Elkhateeb et al. 2019b).

Another fascinating mushroom is *Dictyophora indusiata* which is famous for its medical importance since 618 AD (Han et al. 2017). The extract of *D. indusiata* has been reported to have antimicrobial, and antioxidant activities (Oyetayo et al. 2009; Elkhateeb et al. 2020). Moreover, polysaccharides extracted from *D. indusiata* have great potentiality to be applied in functional food.

In this study, metabolites profiles of n-hexane extracts of the three mushrooms *Metacordyceps neogunnii*, *Hericium erinaceus*, and *Dictyophora indusiata* were elucidated using gas chromatography-mass spectrometry (GC-MS) analysis. At the same time, *in vitro* cholesterol-lowering capabilities, antioxidant activities, and anti-human colon cancer abilities of their n-hexane extracts were investigated.

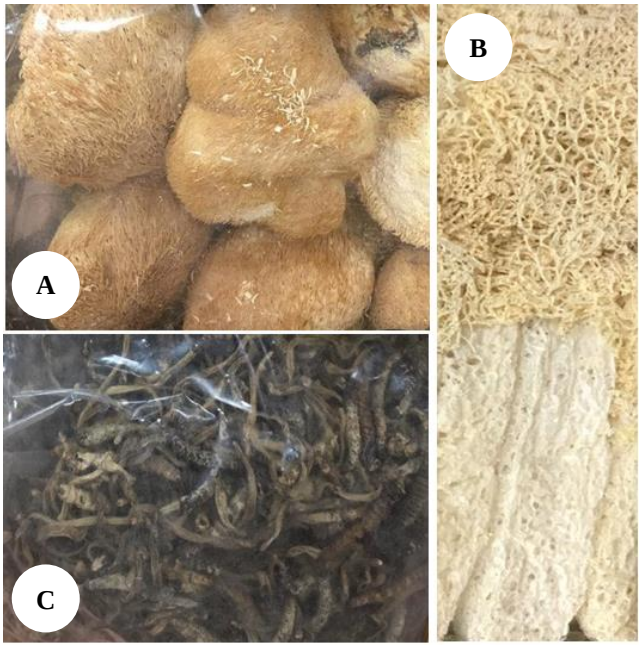

Figure 1. Medicinal mushrooms used in this study: A. *Hericium erinaceus*, B. *Dictyophora indusiata*, and C. *Metacordyceps neogunnii*. Photographs were taken by WAE

MATERIALS AND METHODS

Study area

This study was conducted in the laboratories of the national research center of Egypt. In the departments of chemistry of natural and microbial products, phytochemistry and Plant Systematic, Drug Bioassay-Cell Culture Laboratory, pharmacognosy Department. Also, in the laboratory of engineering Research Center of Southwest Bio-Pharmaceutical Resources, Guizhou University in China.

Sample and extraction

Metacordyceps neogunnii, *Hericium erinaceus* and *Dictyophora indusiata* were kindly supplied by Prof. Dr. Ting-Chi Wen from Guizhou, Tianqi, Wildlife, Sources, Conservation R&D Centre in Guiyang city, Guizhou Province, China (Figure 1).

Two hundred and fifty grams of each mushroom 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 48h prior to filtering. The resulting filtered extracts were concentrated at 37 °C using a rotary evaporator. Obtained extracts were stored at 4ºC in clean closed containers until further use.

GC-MS analysis

The GC-MS analyses of the n-hexane extracts were performed using a gas chromatography-mass spectrometry instrument in the Department of Medicinal and Aromatic Plants Research, National Research Center, Egypt with the following specifications. Instrument: a 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 x 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 ºC for 1 min; rising at 3.0º C /min to 240º C and held for 1 min. The injector and detector were held at 240 ºC. 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 or with those of the Wiley 9 and NIST08 mass spectra library.

Antioxidant scavenging activity of mushrooms n-hexane extracts

DPPH (1-diphenyl-2-picrylhydrazyl) scavenging activity was measured as described previously by Lee *et al.* (2010). Briefly, 500 µl of ethanolic DPPH solution (0.4 mmol) was mixed vigorously with 500 µl of each mushroom extract (80 mg/mL) or water (as a control) and

incubated at 37°C in the dark for 1h. The absorbance of the mixture was measured spectrophotometrically at 517 nm. The scavenging activity was calculated as; Scavenging activity (%) = [1- (A_s - A_b)/A_c] x100, whereas A_b, A_c and A_s is the absorbance of the blank (ethanol and sample), the control (DPPH and deionized water) and the sample (DPPH and sample), respectively. Ascorbic acid at the concentration of 0.1% was used as positive control.

In vitro anti-humann colon cancer activity of mushrooms n-hexane extracts

Cell culture

HCT116 human colon carcinoma 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 plates, left to attach overnight, and then treated with mushrooms extracts for three 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). Cell monolayers were washed with 250 µl PBS. 100 µl of pNPP substrate solution was added per well, then plates were incubated for 4 hours at 37°C. Sodium 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 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:

$$[1- (D/S)] \times 100$$

Where, D and S denote the optical density of drug and solvent treated wells, respectively.

In vitro hypocholesterolemic abilities of mushrooms n-hexane extracts.

Different concentrations of each mushroom extract (0.5-4.0%) were prepared as described previously (Elkhateeb et al. 2019c). Soluble cholesterol (1 mL) was added to 4 mL of each concentration and incubated at room temperature for 24, 48, 72, and 96 hrs. 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 4 mL distilled water to 1 mL soluble cholesterol. The percentage of cholesterol-reducing activity was calculated as describes previously (Pan and Zhang 2005) as follows:

$$\text{Cholesterol reducing activity (\%)} = [(A_0 - A)/A_0] \times 100$$

Whereas A_0: absorbance of the control (500 nm); A: absorbance of the sample (500 nm). Tests were carried out in triplicates.

RESULTS AND DISCUSSION

GC-Ms analysis of the n-hexane extracts of *M. neogunnii, D. indusiata* and *H. erinaceus*

Gc-Ms analyses were performed on the n-hexane extracts of the three mushrooms. As shown in Figure 2, and Table 1, 29 compounds were detected in the n-hexane extract of *Metacordyceps neogunnii*. The total peak areas of the detected compounds were 93.66 %, the probabilities of the structures of the detected compounds are listed in Table 1. The major peak areas are 11.57% Hexadecanoic acid, methyl ester $C_{17}H_{34}O_2$, 15.94% 9,12-Octadecadienoic acid, methyl ester $C_{19}H_{34}O_2$, 50.74 % 9-Octadecenoic acid (Z)-, methyl ester (Oleic acid, methyl ester) $C_{19}H_{36}O_2$, 3.05 % 9,12-Octadecadienoic acid (Z,Z) (Linoleic acid ($C_{18}H_{32}O_2$, for which represented 81.48 % of the total peak. On the other hand, GC-Ms analysis of the n-hexane extract of *Hericium erinaceus* (Figure 3, Table 2) resulted in detection of 33 compounds. The total peak areas of the detected compounds were 90.11 %, the probabilities of the structures of the detected compounds are Pentane, 1-nitro- $C_5H_{11}NO_2$ 18.27% and 1,6-Octadiene, 3,7-dimethyl- $C_{10}H_{18}$, 14.23%, and the major peak areas are for which represented 32.50 % of the total peak areas (Figure 3). Finally, as shown in Figure (4), and Table 3, GC-Ms analysis of the n-hexane extract of *Dictyophora indusiata* resulted in identification of 22 compounds. The total peak areas of the detected compounds are 99.89 %, the probabilities of the structures of the detected compounds are listed in Table 3. The major peak areas are 82.85 % for Butane, 1-isocyano- C_5H_9N, and 10.87 % for Pentane, 1-nitro $C_5H_{11}NO_2$ for which represented 93.72 % of the total peak areas.

Antioxidant scavenging activity of mushrooms n-hexane extracts

The *in vitro* hydrogen-donating or radical scavenging activity of the three mushrooms n-hexane extracts was evaluated using the stable DPPH reagent. As shown in Figure (5), all tested mushrooms extract exerted very good antioxidant capabilities. *D. indusiata* exhibited a relatively higher antioxidant activity (87.8±1.2%) at concentration 80 mg/mL, followed by *H. erinaceus*, then *M. neogunnii* which have recorded 84.9±1.6%, and 77.3±1.3%, respectively at the same concentration.

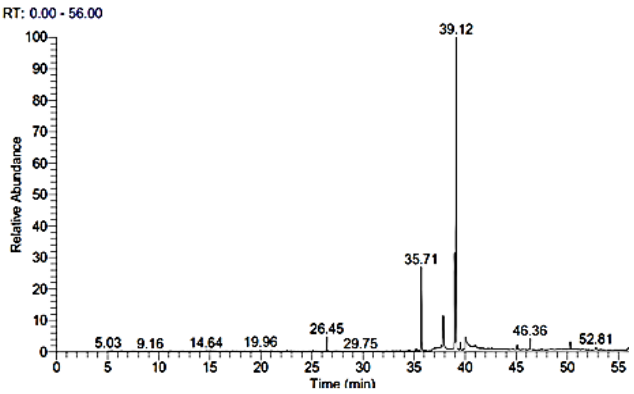

Figure 2. Total ion chromatogram of n-hexane extract of *Metacordyceps neogunnii*

In vitro anti-humann colon cancer activity of mushrooms n-hexane extracts

The cytotoxic activities of the tested mushrooms n-hexane extracts were assessed against HCT116 human colon carcinoma cell line. As shown in Fig (6), the anticancer activity was concentration-dependent. Using *M. neogunnii* extract at concentration of 100 µg/mL resulted in a cytotoxicity of 68.6±3.6% whereas both *D. indusiata* and *H. erinaceus* exhibited weaker cytotoxicity effects using the same concentration (100 µg/mL). Cytotoxicity caused by *D. indusiata* extract was 19.3±3.2% while that resulted from treatment with *H. erinaceus* extract was18.3±1.7%.

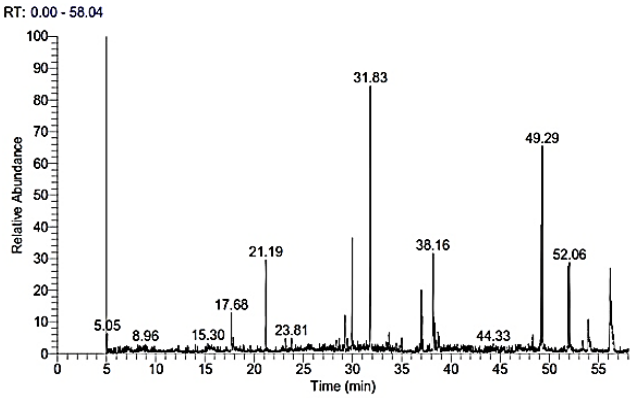

Figure 3. Total ion chromatogram of n-hexane extract of *Hericium erinaceus*

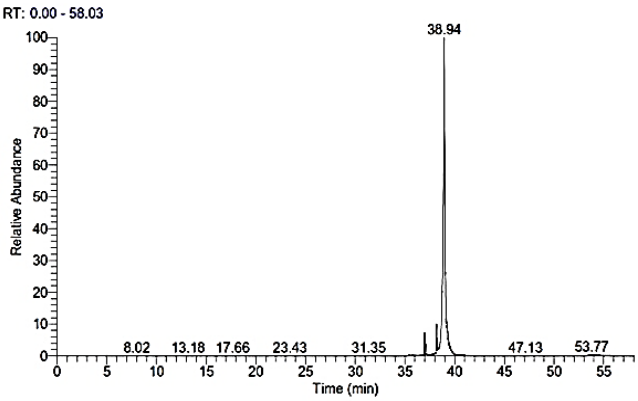

Figure 4. Total ion chromatogram of n-hexane extract of *Dictyophora indusiata*

Table 1. GC/MS analysis of *Metacordyceps neogunnii* n-hexane extract

Peak No.	Rt (min.)	MW	Area %	Molecular formula	Probabilities of the detected compounds
1	26.45	204	2.01	$C_{14}H_{20}O$	(3E)-5-Isopropyliden-6-methyl-3,6,9-decatrien-2-one
2	33.56	256	0.12	$C_{16}H_{32}O_2$	Tetradecanoic acid, 12-methyl-, methyl ester
3	34.55	278	0.26	$C_{16}H_{22}O_4$	1,2-Benzenedicarboxylic acid, dibutyl ester
4	35.15	268	0.45	$C_{17}H_{32}O_2$	9-Hexadecenoic acid, methyl ester, (Z)- (Methyl palmitoleate)
5	35.71	270	11.75	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
6	36.21	468	0.10	$C_{32}H_{52}O_2$	Lup-20 (29)-en-3-ol, acetate, (3á)
7	36.93	490	0.47	$C_{35}H_{70}$	17-Pentatriacontene
8	37.03	310	0.40	$C_{22}H_{46}$	Docosane
9	37.17	280	0.20	$C_{18}H_{32}O_2$	Methyl 9,12-heptadecadienoate
10	37.36	282	0.13	$C_{18}H_{34}O_2$	cis-10-Heptadecenoic acid, methyl ester
11	37.64	284	0.42	$C_{18}H_{36}O_2$	Heptadecanoic acid, methyl ester
12	38.96	294	15.94	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid, methyl ester (Linolelaidic acid, methyl ester)
13	39.12	296	50.74	$C_{19}H_{36}O_2$	9-Octadecenoic acid (Z)-, methyl ester (Oleic acid, methyl ester)
14	39.51	298	1.03	$C_{19}H_{38}O_2$	Octadecanoic acid, methyl ester (Methyl stearate)
15	39.82	328	0.11	$C_{26}H_{16}$	6,8-Bisdehydro-4,5:10.11-dibenzopentatridecafulval-ene
16	40.04	280	3.05	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z,Z) (Linoleic acid)
17	40.96	354	0.79	$C_{25}H_{26}N_2$	8,9,10,11-Tetrahydro-8,8,11,11,15-pentamethylanchraceno [1,2-c]1,8-naphthridin
18	42.28	215	0.20	$C_{12}H_{25}NO_2$	Octanoic acid, 2-dimethylaminoethyl ester
19	42.57	592	0.16	$C_{38}H_{76}N_2O_2$	Octadecanamide, N,N'-1,2-ethanediylbis-
20	42.63	320	0.18	$C_{21}H_{36}O_2$	11,14,17-Eicosatrienoic acid, methyl ester (Methyl 11,14,17-icosatrienoate)
21	43.01	392	0.11	$C_{24}H_{40}O_4$	Cholan-24-oic acid, 3,12-dihydroxy-, (3à,5á,12à) (Deoxycholic acid)
22	44.65	354	0.21	$C_{21}H_{38}O_4$	9,12-Octadecadienoicacid (Z,Z)-,2-hydroxy-1- (hydroxymethyl)ethyl ester (Linolein, 2-mono-)
23	45.08	131	0.82	$C_6H_{13}NO_2$	Acetic acid, 2- (dimethylamino)ethyl ester
24	46.36	540	1.58	$C_{30}H_{36}O_9$	8'-O-Ethyl-á-alectoronic acid
25	47.48	314	0.24	$C_{21}H_{30}O_2$	Delta.-1 (2)- tetrahydrocannabinol
26	49.22	378	0.21	$C_{27}H_{54}$	Cyclohexane,1,3,5-trimethyl-2-octadecyl-
27	50.31	410	1.20	$C_{30}H_{50}$	Squalene
28	50.78	363	0.16	$C_{21}H_{17}NO_3S$	2-[(2'-Acetylphenyl)amino]-5-[(4''-methylphenyl)thio]-benzo[b]- (1,4)-quinone
29	52.81	376	0.62	$C_{24}H_{24}S_2$	Bis (6-methylthieno[3',2, 8,9]bicyclo[4.3.0]non- 1 (2)-en-3-ylidene)

Note: R_t: Retention time, MW: Molecular weight

Table 2. GC/MS analysis of n-hexane extract of *Hericium erinaceus*

Peak No.	Rt (min.)	MW	Area %	Molecular formula	Probabilities of the detected compounds
1	17.69	100	3.42	$C_6H_{12}O$	Butanal, 2-ethyl-
2	20.58	168	4.39	$C_{11}H_{20}O$	1,7-Nonadien-4-ol, 4,8-dimethyl-
3	23.20	172	0.72	$C_{10}H_{20}O_2$	Octanal, 7-hydroxy-3,7-dimethyl-
4	23.81	378	0.73	$C_{27}H_{54}$	Cyclohexane, 1,3,5-trimethyl-2-octadecyl-
5	28.33	186	0.80	$C_{11}H_{22}O_2$	1-Hydroxyundecan-10-one
6	28.66	126	0.50	C_9H_{18}	3-Heptene, 2,6-dimethyl-
7	29.21	115	1.95	$C_5H_9NO_2$	3-Methyl-3-nitrobut-1-ene
8	29.44	115	0.71	$C_5H_9NO_2$	1-Pentene, 5-nitro-
9	29.96	112	7.02	$C_7H_{12}O$	Allyl methallyl ether
10	31.38	744	0.42	$C_{16}H_8Br_6Cl_2$	(Z) and (E) à,á-Dibromo-3,3'-dichloro- 4,4'-bis (dibromomethyl) stilbene
11	31.82	138	14.23	$C_{10}H_{18}$	1,6-Octadiene, 3,7-dimethyl-
12	33.46	662	0.54	$C_{39}H_{42}N_4O_6$	34,38-Dioxo-33,35,36,37-tetramethoxy-31- methyl-3,7,23,27-tetraaza heptacyclo (27.3.1.1.1.11.1)octaconta-1 (33),8- (35),9,11,13 (36),14,16,18 (37),19,21,29,31-dodecaene
13	33.70	144	1.08	$C_8H_{16}O_2$	Heptanoic acid, methyl ester
14	34.44	140	0.42	$C_9H_{16}O$	trans,-2,6-Nonadien-1-ol
15	35.00	186	0.72	$C_{11}H_{22}O_2$	Nonanoic acid, ethyl ester
16	36.95	138	3.26	$C_{10}H_{18}$	Bicyclo [7.1.0]decane
17	37.07	140	1.78	$C_{10}H_{20}$	1-Octene, 3,7-dimethyl-
18	37.18	131	0.46	$C_6H_{13}NO_2$	Hexane, 1-nitro
19	38.16	138	4.51	$C_{10}H_{18}$	1-Decyne
20	38.25	142	1.46	$C_9H_{18}O$	2-Nonen-1-ol, (E)-
21	38.36	126	1.14	C_9H_{18}	1-Nonene
22	38.51	191	0.61	$C_9H_9N_3O_2$	1H-1,2,3-Triazole-4-carboxylic acid
23	38.67	117	1.54	$C_5H_{11}NO_2$	Pentane, 1-nitro-
24	38.82	131	0.94	$C_6H_{13}NO_2$	Hexane, 1-nitro-
25	41.92	184	0.44	$C_{12}H_{24}O$	Z-2-Dodeceno
26	44.79	73	0.53	$C_4H_{11}N$	Isobutylamine
27	46.75	118	0.47	$C_6H_{14}O_2$	Hydroperoxide, hexyl
28	48.32	112	0.75	C_8H_{16}	1-Hexene, 3,3-dimethyl-
29	49.15	117	18.27	$C_5H_{11}NO_2$	Pentane, 1-nitro-
30	52.06	118	4.02	$C_6H_{14}O_2$	Hydroperoxide, 1-methylpentyl
31	53.39	102	7.47	$C_6H_{14}O$	3-Methyl-3-pentanol
32	56.31	118	4.37	$C_6H_{14}O_2$	Hydroperoxide, 1-methylpentyl
33	56.52	119	0.44	$C_4H_9NO_3$	1-Butanol, 2-nitro

Note: R_t: Retention time MW = Molecular weight

Table 3. GC/MS analysis of n-hexane extract of *Dictyophora indusiata*

Peak No.	Rt min.)	MW	Area %	Molecular formula	Probabilities of the detected compounds
1	11.82	100	0.07	$C_6H_{12}O$	Butanal, 2-ethyl-
2	21.16	102	0.04	$C_6H_{14}O$	1-Pentanol, 2-methyl
3	31.60	116	0.04	$C_6H_{12}O_2$	Hexanoic acid
4	33.68	130	0.11	$C_7H_{14}O_2$	Hexanoic acid, methyl ester
5	34.99	186	0.05	$C_{11}H_{22}O_2$	Nonanoic acid, ethyl ester
6	35.39	284	0.22	$C_{18}H_{36}O_2$	Valeric acid, 4-tridecyl ester
7	36.96	117	10.47	$C_5H_{11}NO_2$	Pentane, 1-nitro
8	37.06	131	4.63	$C_6H_{13}NO_2$	Hexane, 1-nitro-
9	37.76	83	82.85	C_5H_9N	Butane, 1-isocyano-
10	37.97	117	0.19	$C_5H_{11}NO_2$	Amyl nitrite
11	38.35	102	0.15	$C_6H_{14}O$	Hexyl alcohol
12	39.87	102	0.08	$C_6H_{14}O$	3-Methyl-3-pentanol
13	40.78	160	0.04	$C_8H_{16}O_3$	5-Hexyl-1,2,4-trioxalane
14	43.13	86	0.05	$C_5H_{10}O$	Cyclobutane, methoxy-
15	43.19	115	0.05	$C_6H_{13}NO$	2-Pentanone, 4-amino-4-methyl-
16	43.46	209	0.05	$C_{13}H_{23}NO$	(Z)-6- (N,N-Diallylamino)-2-methyl-4-hexen-3-ol
17	46.97	142	0.05	$C_5H_6N_2O_3$	3,5-DIMETHYL-4-OXO-4H-PYRAZOLE1,2-DIOXIDE
18	47.07	671	0.05	$C_{29}H_{39}Br_2NO_7$	1-Acetyl-4,4-bis[4- (3-bromopropoxy)-3,5-dimethoxyphenyl]piperidine
19	47.51	694	0.06	$C_{48}H_{62}N_4$	2,7,12,17-Tetraethyl-3,5:8,10:13,15:18,20-tetrakis (2,2-dimethylpropano) porphyrin
20	49.14	114	0.05	$C_8H_{16}O_2$	5-Methyl-2-methylenehexane-1,4-diol
21	52.47	119	0.45	$C_4H_9NO_3$	1-Butanol, 2-nitro-
22	53.38	130	0.14	$C_8H_{18}O$	4-Methyl-3-heptanol

Note: Rt: Retention time, MW: Molecular Weight

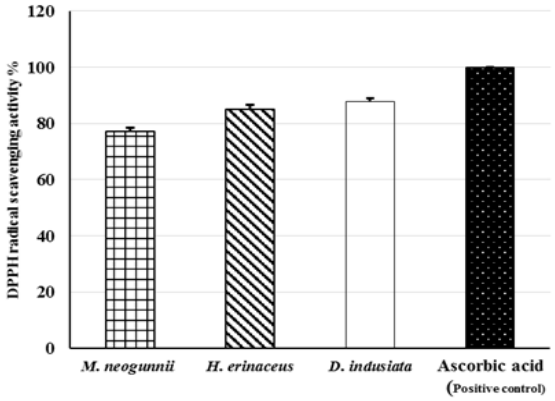

Figure 5. DPPH radical scavenging activity of n-hexane extracts of *H. erinaceus, M. neogunnii,* and *D. indusiata* at concentration 80 mg/mL. Ascorbic acid at concentration 0.1% was used as positive control. Each value represents the mean of three replicates (Mean ±SD)

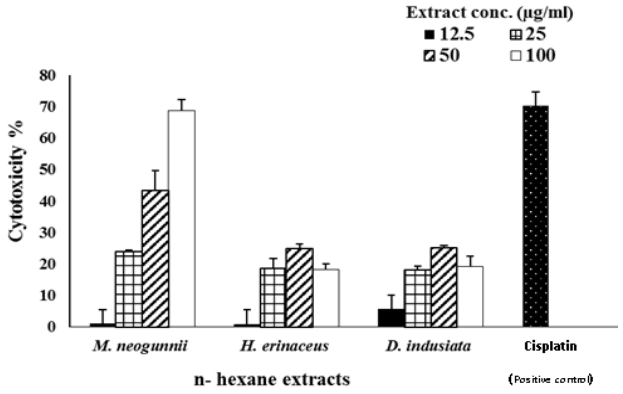

Figure 6. Cytotoxicity % of *M. neogunnii, H. erinaceus,* and *D. indusiata* n-hexane extract against HCT116 cell line monolayers. Cisplatin at concentration 50 µM was used as positive control. Each value represents the mean of three independent experiments (Mean ±SD)

***In vitro* hypocholesterolemic activities of n-hexane mushrooms extracts.**

The effect of *D. indusiata H. erinaceus,* and *M. neogunnii* n-hexane extracts on cholesterol concentration was evaluated using different concentrations of extracts and under incubation periods that reached 4 days at room temperature. The results shown in Table 4 revealed that the cholesterol reduction activities (CRA %) of the three extracts is depending on the extract concentration as well as the incubation period. All extracts exerted potent *in vitro* cholesterol concentration lowering capabilities. Results ranged from 72.9±0.9% to 100±0% in case of *M. neogunnii* n-hexane extract, from 78.6±1.1% to 100±0% by using *H. erinaceus* n-hexane extract, and from 77.9±1.1% to 100±0% when *D. indusiata* n-hexane extract was used.

By finishing the first 24h, both extracts of *M. neogunnii* and *D. indusiata* at concentration of 32 mg/mL caused a higher reduction in cholesterol concentration (89.3±1.3%; and 89.3±1.7%, respectively), in comparison with *H. erinaceus* extract which exhibited 88.6±1.4%. Order of extracts potency using the same concentration (32 mg/mL) after 48h were totally different, *H. erinaceus* extract came first recording 92.1±1.6%, then *M. neogunnii* extract (91.1±1.1%) followed by *D. indusiata* extract (90.5±1.5%). By the third day, *H. erinaceus* extract continued its superiority over other extracts by recording a cholesterol-reducing activity of 95.7±1.2%, in comparison with *M. neogunnii* and *D. indusiata* extracts (92.9±1.3%, and 91.4±1.4%) respectively. A promising total reduction in cholesterol concentration was achieved by the three extracts by the end of 96h.

Discussion

Many serious diseases such as diabetes, neurodegenerative, and cardiovascular diseases are developed pathologically by some free radicals. Generally, free radicals are generated as natural by-products from normal cellular metabolism of oxygen. Additionally, they can be induced by many factors like smoking, diet, lifestyle, stress, UV light, and ionizing radiation (Katerji et al. 2019). Free radicals are responsible for damaging proteins, membranes, and nucleic acids through several biological processes which result in many diseases (Bhattacharyya et al. 2014; Jiang et al. 2016). Moreover, there is a well-established relation between free radicals and cancer diseases (Sammar et al. 2019) which are major health challenges that threaten lives and rise annual mortalities worldwide. Hence, there is a serious need for screening for potent sources rich in antioxidants, which act as defense systems that regulate the levels of these free radicals. On the other hand, high cholesterol level is associated with an elevated risk of cardiovascular diseases **(**Elkhateeb et al. 2019b**)**. Therefore, finding hypocholesterolemic potent sources are also of great need. Obviously, prevention and treating of such lethal diseases using natural edible sources would be of great significance to human health.

In the present study, it was aimed to compare the biological potencies of the mushrooms *Hericium erinaceus, Metacordyceps neogunnii,* and *Dictyophora indusiata,* through evaluating the *in vitro* antioxidant, anti-colon cancer and hypocholesterolemic properties of their n-hexane extracts. These three mushrooms are edible mushrooms that are consumed for their beneficial nutritional characteristics.

Antioxidant properties of n-hexane extracts were investigated by testing their DPPH radical scavenging activities at 80 mg/mL concentration. All extracts showed promising antioxidant activities, which can be attributed to the richness of these extracts in fatty acid methyl esters which are known for exhibiting antioxidant activities (Pinto et al. 2017). Many studies had mentioned the antioxidant activities of the extracts of *H. erinaceus* (Charumathy et al. 2017; Lew et al. 2019); and *D. indusiata* (Oyetayo et al. 2009; Zhang et al. 2016).

Table 4. *In vitro* cholesterol-reducing activity (CRA %) of the n-hexane extracts of *Metacordyceps neogunnii, Hericium erinaceus,* and *Dictyophora indusiata*

Extract conc. (mg/mL)	CRA%											
	***M. neogunnii* extract**				***H. erinaceus* extract**				***D. indusiata* extract**			
	Incubation time (h)											
	24	**48**	**72**	**96**	**24**	**48**	**72**	**96**	**24**	**48**	**72**	**96**
4	72.9±0.9	74.5±1.7	77.1±1.2	89.3±1.3	78.6±1.1	79.0±0.9	79.3±0.8	89.3±1.9	77.9±1.1	79.1±0.8	80.0±0.4	89.3±0.7
8	78.6±1.8	79±1.3	80.0±1.2	95.7±1.8	80.7±1.3	81.0±1.0	81.4±0.6	93.6±1.1	79.3±0.8	80.4±1.4	81.4±1.4	91.4±0.6
16	82.1±1.6	83.1±1.8	83.6±2.1	96.4±0.6	83.6±1.4	84.3±0.9	85.0±0.8	95.0±0.8	85.0±0.8	86.6±1.1	87.9±1.4	95.7±1.1
24	85.7±0.9	86±0.7	86.4±1.5	99.3±0.3	84.3±1.3	85.7±1.3	87.1±0.9	95.7±1.2	87.9±1.2	89.3±1.3	90.7±1.3	98.6±0.6
32	89.3±1.3	91.1±1.1	92.9±1.3	100.0±0	88.6±1.4	92.1±1.6	95.7±1.2	100.0±0	89.3±1.7	90.5±1.5	91.4±1.4	100.0±0

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

On the other hand, evaluating the anti-colon cancer activities of the extracts revealed potency of *M. neogunnii extract* at concentration of 100 µg/mL in comparison with other extracts at the same concentrations. Several *in vivo* and *in vitro* studies have described the anticancer activities of *D. indusiata* extracts against group of human cancer cell lines (Liao et al. 2015; Han et al. 2017), also the anticancer activities of extract is well known (Kim et al. 2013; Li et al. 2014; Am, 2017). The *in vitro* hypocholesterolemic studies which were conducted on the three extracts showed their promising capabilities in cholesterol-reducing. All extracts succeeded in lowering cholesterol concentrations in 100% after incubation for 4 days at room temperature. At the third day, *M. neogunnii* n-hexane was the fastest in reaching the highest reducing ability by recording 99.3±0.3%. It was previously reported that many medium-chain fatty acids as those detected in the extracts are capable of reducing cholesterol levels (Liu et al. 2017). It should be noted that studies on antioxidant, anti- colon cancer, and hypocholesterolemic activities of *M. neogunnii* extract are relatively rare, and this is the first study that describes its antioxidant activities.

Chemical analyses on the different extracts were performed in order to describe the metabolic profiles of the extracts, which may help in understanding the secondary metabolites originated from those mushrooms and their role in the observed biological activities. Medium-chain fatty acid such as caproic acid, which was detected in the n-hexane extract of *D. indusiata* has significant anticancer and anti-proliferation activities against human colorectal, skin and breast cancer cells and could potentially be used to prevent and/or treat these cancers (Narayanan et al. 2015). Compound such as Cyclohexane, 1,3,5-trimethyl-2-octadecyl- which was detected in *H. erinaceus* extract has anticancer activity (Viswanathan, 2014). In general, results in our study suggested an overall superiority of *M. neogunnii extract.* GC-Ms analysis of *M. neogunnii* n-hexane extract revealed the presence of 13 ester compounds out of the 29 detected compounds which came in accordance with results described by She et al (2019). Promising biological activities exerted by *M. neogunnii* extract can be explained by the presence of many bioactive compounds representing major peak areas detected by GC-Ms analysis (Table 1). Palmitic acid methyl ester (representing 11.75% of total peak area) is known for its antioxidant, hypocholesterolemic activities among many other activities (Mujeeb et al. 2014). On the other hand, 9 Octadecenoic acid (Z), methyl ester which was detected as major peak (50.74%) in *Metacordyceps* extract has strong anticancer and antioxidant activities (Yeong et al. 1989; Asghar et al. 2011; Belakhdar et al. 2015). Moreover, 9 Octadecenoic acids (Z), methyl ester exhibits hypocholesterolemic effect (Krishnamoorthy and Subramaniam, 2014). Another ester (9, 12- octadecadienoic acid (Z, Z)-, methyl ester) was detected in this extract (representing 15.94% of total peak area) is known for its antioxidant and anticancer properties (Kumar et al. 2010; Wei et al. 2011). Finally, 9,12 Octadecadienoic acid (Z,Z) (Linoleic acid (which was detected in 3.05 % peak area is also characterized by having promising antioxidant activities (Peyrat-Maillard et al. 2003; Rahman et al. 2014). Furthermore, Dommels et al (2003) described the effect of high doses of linoleic acid on inhibiting colon cancer cell line Caco-2 proliferation. In another study, a protective effect against cancer development was accomplished by high intake of fatty acids such as linoleic acid (Horrobin and Ziboh, 1997). A synergism between those different compounds can explain the recorded promising biological activities of those mushrooms extracts.

In conclusion, the present study provides encouraging data for using extracts of *H. erinaceus*, *M. neoqunnii*, and *D. indusiata* in the pharmaceutical industry, and for disease prevention. An advantage that facilitates doing that is the fact that all tested mushrooms in this study are already edible mushrooms which also support their use as functional foods. Additionally, the broad therapeutic values of these mushrooms reserve promising future for them in modern alternative medicine. Further studies are encouraged to understand the mode of action behind the properties of their bioactive compounds, and to explain possible synergism between such compounds which resulted in these promising results. Furthermore, finding a natural edible source having multiple biological activities against different life-threatening diseases as colon-cancer, cardiovascular diseases caused by high cholesterol levels, as well as exhibiting promising antioxidant properties is this era target in order to win the human battle against such life-threatening diseases.

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