GC-MS analysis and *in-vitro* hypocholesterolemic, anti-rotavirus, anti-human colon carcinoma activities of the crude extract of a Japanese *Ganoderma* spp

Waill A. Elkhateeb[a], Ghoson M. Daba[a], Donia Sheir[a], Asmaa Negm El-Dein[a], Walid Fayad[b], ELmahdy M. Elmahdy[c], Mohamed N.F. Shaheen[c], Paul W. Thomas[d,e], Ting-Chi Wen[f]

[a]Chemistry of Natural and Microbial Products Department, Pharmaceutical Industries Researches Division, [b]Drug Bioassay-Cell Culture Laboratory, Pharmacognosy Department, [c]Environmental Virology Laboratory, Water Pollution Research Department, Environmental Research Division, National Research Centre, Dokki, Egypt, [d]Mycorrhizal Systems Ltd, Lancashire, [e]University of Stirling, Stirling, UK, [f]The Engineering Research Center of Southwest Bio-Pharmaceutical Resources, Ministry of Education, Guizhou University, Guiyang, China

Correspondence to Ghoson M. Daba, PhD, Chemistry of Na tural and Microbial Products Department, National Research Centre, Dokki, Giza 12622, Egypt. Tel: +201013241936; fax: +20233370931; e-mail: ghoson.daba@yahoo.com

Received 25 November 2018
Accepted 2 January 2019

Egyptian Pharmaceutical Journal 2019, 18:102–110

Background and objective
Medicinal mushrooms are mines of various biologically active compounds. Therefore, chemical analysis and *in-vitro* evaluation of some biological activities of the Japanese originated mushroom *Ganoderma* spp. were conducted.
Materials and methods
Extraction of the fruiting bodies of *Ganoderma* spp. was accomplished using 80% methanol. This extract was investigated for its *in-vitro* cholesterol-lowering activity, anti-rotavirus effect, and anti-human colon cancer influence. Moreover, a gas chromatography–mass spectrometry analysis for this extract was performed.
Results and conclusion
The gas chromatography–mass spectrometry analysis resulted in the detection of 39 compounds, which were generally saturated and unsaturated fatty acids, and alkenes. The crude extract exhibited a promising *in-vitro* cholesterol-lowering activity (100±0%) after 96 h of incubation at room temperature. The same crude extract showed a moderate anti-rotavirus SA-11 strain effect with a therapeutic index of 9.3. Moreover, *Ganoderma* spp. extract displayed a strong activity toward HCT116 human colon carcinoma cell line, resulting in a cytotoxicity of 84.03±0.93% on HCT116 cell line monolayers. *Ganoderma* spp. crude extract represents a promising source of biologically active compounds that could by further investigations represent support and/or alternative to the currently used drugs.

Keywords:
biological activity, *Ganoderma*, gas chromatography–mass spectrometry, human colon cancer, hypocholesterolemic activity, rotavirus

Egypt Pharmaceut J 18:102–110

1687-4315

Introduction

According to the world health organization (WHO), about 17.7 million people die annually from cardiovascular diseases (CVDs), which represents about 31% of mortalities worldwide [1,2]. CVD is associated with hypercholesterolemia, atherosclerosis, and lactate dehydrogenase oxidation. Hence, regulating or lowering the cholesterol level is the key factor in the treatment and prevention of CVD.

Lovastatin and its analogs are famous cholesterol-lowering agents, commonly referred to as statins, which act as inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA reductase [3]. Despite their widespread use within the population, they are not without risk. It is broadly accepted that contraindications and interactions with certain foods exist, but further there are many side effects reported from statin use and these may be severe enough to require immediate dose reduction or cessation of medication. These statin-associated symptoms include diabetes mellitus, statin-associated muscle symptoms, and central nervous system complaints [4]. Such serious side effects, along with contraindications and interactions, present the need to identify and develop novel cholesterol-lowering compounds other than statins.

Rotavirus is a highly contagious infectious agent causing high rates of mortalities in developing countries, especially among newborns, infants, and young children [5,6]. According to the WHO reports, each year about 450 000 children under 5 years of age die because of diarrhea caused by rotavirus [7]. Till now, no drugs are available to treat rotavirus nor to prevent the diarrhea resulting from it [8]. The widespread existence and frequent epidemics of this dangerous virus encourage a rapid

 DOI: 10.4103/epj.epj_50_18

search for natural, effective, and safe compounds that exhibit a therapeutic effect toward rotavirus.

Worldwide, colorectal cancer (also termed colon cancer) is the third most commonly diagnosed cancer, after lung and breast cancers. Also, it represents the second biggest cause of cancer deaths, resulting in about 862 000 deaths annually, according to the WHO report [9]. Therefore, there is a critical need to identify further compounds that may provide effective activity against such lethal diseases.

Medicinal and edible mushrooms are natural sources of various compounds, and are used in Asian traditional medicine from the millennia as a medicinal supplementary food to treat and prevent numerous diseases [10]. Many studies have investigated the pharmaceutical characteristics of certain fungal species including their activities such as antimicrobial, antiviral, anticancer, anti-inflammatory, immunomodulating, hypocholesterolemic, hypoglycemic, antiatherogenic, and hepatoprotective agents [11–16].

Ganoderma is a genus that includes about 80 species, and belongs to the family Ganodermataceae [17]. *Ganoderma* has been used from centuries in traditional oriental medicine and specifically in Japan, China, and Korea [18]. Currently, *Ganoderma* is available worldwide as a food supplement. Whole *Ganoderma* or their crude extracts have been intensively investigated for their anti-inflammatory effect [19].

In this study, a gas chromatography–mass spectrometry (GC–MS) analysis of the 80% methanolic extract of the fruiting bodies of a Japanese originated *Ganoderma* spp. was performed. Moreover, different concentrations of this extract were investigated for their *in-vitro* cholesterol-reducing activity (CRA) after different incubation times. The antiviral effect of *Ganoderma* spp. extract was also investigated toward rotavirus SA-11 strain. Finally, the same extract was examined for its anticancer activity against HCT116 human colon carcinoma cell line.

Materials and methods

Collection and identification of the mushroom

The mature mushroom fruiting bodies were found growing in the wild, on the decaying wood of a Japanese cherry (sakura) tree (*Prunus serrulata*) within a park in Chihaya, Higashi ward, Fukuoka Prefecture, Japan. The fruiting body was removed and identified as belonging to the *Ganoderma* genus according to the classification criteria described in the comprehensive guide of the mushroom identification book [20].

Extraction of the metabolites from the mushroom

Approximately 250 g of *Ganoderma* spp. fruiting bodies were washed with distilled water, air dried, and then cut into small pieces and placed in an Erlenmeyer flask containing 80% methanol at room temperature and kept overnight before filtering. The resulting filtered extract was concentrated at 37°C using a rotary evaporator. The obtained extract was stored at 4°C in a clean closed container until further use.

GC–MS analysis

The analysis of the *Ganoderma* spp. crude extract was performed using a GC–MS instrument (TRACE GC Ultra Gas Chromatographs; THERMO Scientific Corp., Waltham, Massachusetts, USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer, Thermo Scientific, San Jose, California, USA). The GC–MS system was equipped with a TG-WAX MS column (30 m×0.25 mm daily, 0.25-µm film thickness). Analysis was 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 the detector were held at 240°C. Diluted samples (1 : 10 chloroform, v/v) of 0.2 µl of the mixtures were always injected automatically in the splitless mode. Mass spectra were obtained by electron ionization 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 [21] and/or with those of the Wiley 9 and NIST08 mass spectral libraries.

In-vitro cholesterol reduction assay

Overall, 0.4 g of the methanolic extract of *Ganoderma* spp. was dissolved in 5 ml distilled water; then different dilutions of this mixture were prepared as illustrated in Table 1. After that, mixtures were supplemented with 1 ml of soluble cholesterol to bring the total volume to 5 ml. These different mixtures were incubated at room temperatures for 24, 48, 72, and 96 h. Cholesterol assay was then performed using the cholesterol assay kit (Biodiagnostic, Cairo, Egypt) to determine the residual amount of cholesterol in the spent broth. A measure of 4 ml of distilled water supplemented with

Table 1 Preparation of different concentrations of *Ganoderma* spp. crude extract mixture for cholesterol-reducing activity (CRA) assay

Extract concentrations	Extract added in ml (its equivalent weight in g)	Distilled water added in ml	Cholesterol solution added in ml
5	0.25 (0.02)	3.75	1
5	0.5 (0.04)	3.5	1
5	1.0 (0.08)	3.0	1
5	1.5 (0.12)	2.5	1
5	2.0 (0.16)	2.0	1

1 ml of soluble cholesterol was used as a control. Finally, the percentage of cholesterol-reducing activity (CRA) was calculated as described previously [22] as follows:

$$\text{Cholesterol reducing activity}\,(\%) = \left[\frac{(A_0 - A)}{A_0}\right] \times 100.$$

where A_0 is the absorbance of the control (500 nm) and A is the absorbance of the sample (500 nm). Tests were carried out in triplicate.

XXXAntiviral activity of crude extract against rotavirus SA-11

Cell lines and virus titration

The Rhesus monkey kidney cell line (MA 104) was used in this study for culturing of the simian rotavirus SA-11 strain. MA 104 cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM). The media were supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 µg/ml streptomycin and 100 units/ml penicillin, and 1% HEPES (4-2-hydroxyethyl-1-piperazineethanesulfonic acid). The cell culture was then incubated under humidified 5% CO_2 atmosphere in CO_2 incubator. The medium used for both cytotoxicity and antiviral assays was containing only 2% of FBS. 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.* [23]. The viral titers were calculated as $TCID_{50}$/0.1 ml (50% tissue culture infectious doses/0.1 ml) according to Spearman–Kärber formula [24]. RV SA-11 stock was kept in small aliquots at −80°C until use.

Cytotoxicity assay

Different concentrations from the *Ganoderma* spp. methanolic crude 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 activities of the tested extract was examined onto MA 104 by using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [25]. 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 included using only the medium. The treated or nontreated cells were incubated for 2 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 a CO_2 incubator. After that, MTT solution was removed and replaced by 50 µl DMSO/well. After 30 min at 37°C, the optical densities were measured using an enzyme-linked immunosorbent assay reader at 540 nm. The percentage of cytotoxic effects was calculated as $[(C–TC)\times100]$, where C and T refer to the optical densities of cell control and treated cells, respectively.

XXXAntiviral activity of crude extract on RV SA-11 by the MTT method

MA 104 cells at a concentration of 5×10^4 cells/well were cultured for 24 h in a CO_2 incubator at 37°C in 96-well microtiter plates. After removing the culture media, three nontoxic concentrations of the crude extract were tested against viral infections. A measure 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 humidified 5% CO_2 atmosphere for 1 h at 37°C and then the mixed solution was added to cell monolayers. After 1 h in CO_2 incubator, the mixed solution was removed. The cell lines were washed two times with a culture medium and then 200 µl of infectious medium (FBS free DMEM containing 2 µl of trypsin) was added to the cells. Virus controls, containing the virus suspension, and cell controls, containing culture 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 and then measured by the MTT as described above. The percentage protection was calculated as $[(T–V)/(C–V)\times100]$, 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 extract was calculated as ratio CC_{50} over IC_{50}.

Effect of crude extract on HCT116 human colon carcinoma cell lines

Cell culture

HCT116 colon carcinoma human tumor cell lines were cultured in 95% humidity, 5% CO_2 at 37°C<AQ: Pls

check whether the change retains the intended meaning>. The cell line was maintained in McCoy's 5 A medium supplemented with 10% FBS.

Cytotoxicity assay

The acid phosphatase assay was used to assess cytotoxicity according to the method described by Yang *et al.* [26]. Overall, 10 000 cells were seeded per well in 96-well 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 *p*-nitrophenyl phosphate (cat. no. N2765; Sigma, Darmstadt, Germany) was dissolved in 10 ml buffer solution (0.1 mol/l sodium acetate, 0.1% triton X-100, pH=5). Cell monolayers were washed with 250 µl PBS. One hundred microliter of pNPP substrate solution was added per well, then the plates were incubated for 4 h at 37°C. Ten microliter of 1 N sodium hydroxide stop solution was added per well. Absorbance was measured directly at a wavelength of 405 nm. All samples were tested in triplicates, and 0.5% DMSO was used as negative control and 50 µmol/l cisplatin was used as positive control. The sample was tested at serial dilutions with a final concentration of 400, 200, 100, 50, 25, 12.5, and 6.25 µg/ml. Percent cytotoxicity was calculated by the formula

$$\left[1-\left(\frac{D}{S}\right)\right]\times 100,$$

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

Results

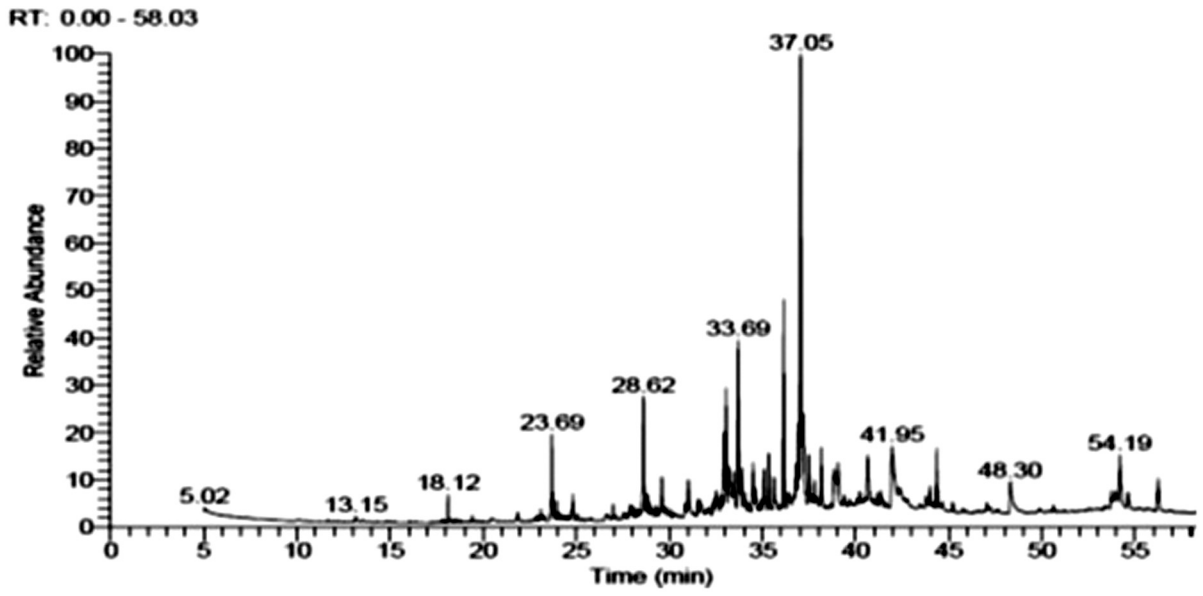
GC/MS analysis

As shown in the chromatogram in Fig. 1, GC–MS analysis of the crude extract of *Ganoderma* spp. showed the presence of about 60% oxygenated compounds and 40% nonoxygenated compounds. Most of the compounds (listed in Table 2) were alkenes, saturated and unsaturated fatty acids, such as pentadecane; hexadecane; octadecane; eicosane; tricosane; decosane; pentacosane; heneicosane; 11-(1-ethylpropyl); methyl-18-methylnonadecanoate; 17-pentatriacontene; (*Z*)-9-octadecenamide; tetratetracontane; docosanoic acid methyl ester; 3-nitro-1,2-benzenedicarboxylic acid;, 1-heptatriacotanol), tricosanoic acid, methyl ester, decosane; 2, 6, 10, 14, 18, 22-tertacosahexaene; 2, 6, 10, 15, 19, 23-hexamethyl; and others.

Hypocholesterolemic activities of the *Ganoderma* spp. methanolic crude extract

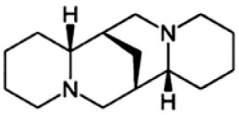
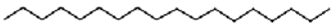
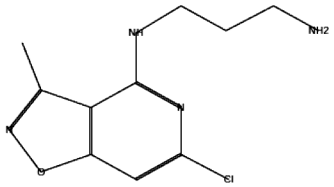
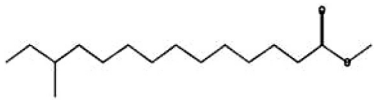
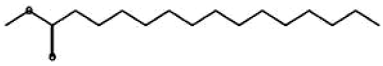
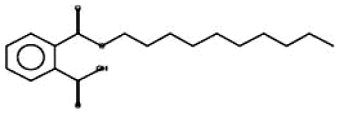
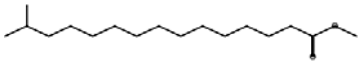
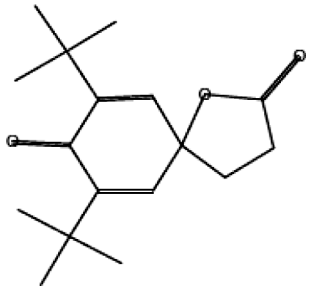
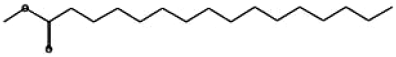
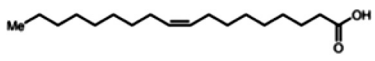
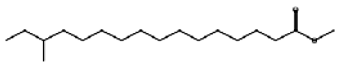
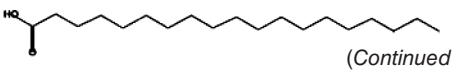
The results shown in Table 3 showed that the methanolic extract of *Ganoderma* spp. exhibited high cholesterol reduction activity *in vitro* with results ranging from 35.1±1.51 to 63.5±1.06% after 24 h, from 54.2±0.95 to 77.3±0.60% after 48 h, from 72.6±1.85 to 90.5±1.05% after 72 h, and from 83.4 ±1.93 to 100%±0 after 96 h depending on the concentration of the extract. The highest cholesterol-reducing activity of *Ganoderma* spp. was achieved after 96 h of incubation by using concentration 5, which is equivalent to using 32 mg/ml of the methanolic crude extract.

Figure 1



Gas chromatography–mass spectrometry chromatogram for the methanolic extract of *Ganoderma* spp. fruiting bodies.

Table 2 List of compounds identified from the methanolic extract of *Ganoderma* spp. by GC–MS analysis

Peak number	Retention time (min)	Molecular formula	Molecular weight	Compound	Structure
1	19.42	$C_{15}H_{32}$	212	Pentadecane	
2	20.45	$C_{15}H_{26}N_2$	234	Sparteine	
3	23.09	$C_{16}H_{34}$	226	Hexadecane	
4	23.69	$C_{18}H_{38}$	254	Octadecane	
5	24.08	$C_{20}H_{42}$	282	Eicosane	
6	28.62	$C_{27}H_{56}$	380	Heptacosane	
7	29.60	$C_{25}H_{52}$	352	Pentacosane	
8	29.68	$C_{10}H_{13}ClN_4O$	240	*N′*(4-Chloro3methyli soxazolo[4,5c] pyridin4yl) propane1,3diamine	
9	30.85	$C16H_{32}O_2$	256	Tetradecanoic acid, 12-methyl-, methyl ester	
10	31.52	$C_{23}H_{48}$	324	Tricosane	
11	31.65	$C_{16}H_{32}O_2$	256	Pentadecanoic acid, methyl ester	
12	32.53	$C_{18}H_{26}O_4$	306	2-(Decyloxycabonyl) benzoic acid	
13	32.93	$C_{17}H_{34}O_2$	270	Pentadecanoic acid, 14-methyl-, methyl ester	
14	33.48	$C_{17}H_{24}O_3$	276	7,9-Di-tert-butyl-1-oxas piro[4.5] deca-6,9-diene-2,8-dione	
15	33.69	$C_{17}H_{34}O_2$	270	Palmitic acid, methyl ester	
16	34.61	$C_{18}H_{34}O_2$	282	Oleic acid	
17	35.07	$C_{18}H_{36}O_2$	284	Hexadecanoic acid, 14-methyl-, methyl ester	
18	35.63	$C_{34}H_{70}$	478	Tetratriacontane	
19	36.37	$C_{19}H_{38}O_2$	298	Nonadecanoic acid	

(*Continued*)

Table2 (*Continued*)

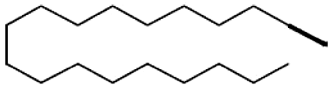
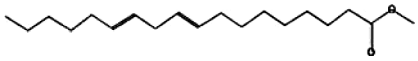
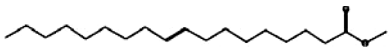
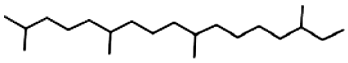
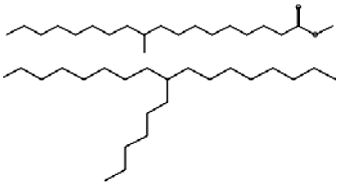
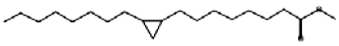
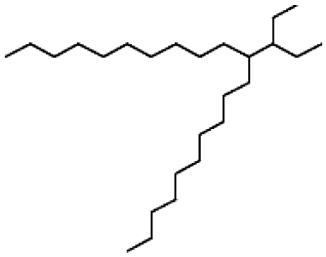
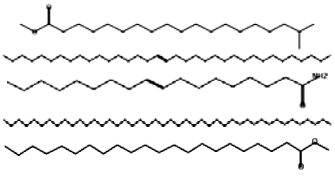
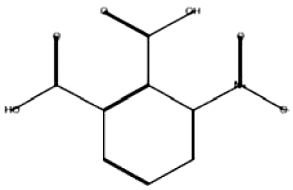
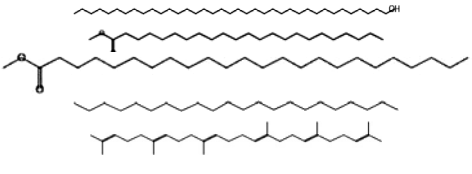
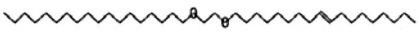
Peak number	Retention time (min)	Molecular formula	Molecular weight	Compound	Structure
20	36.80	$C_{13}H_{33}N$	263	Oleanitrile	
21	36.92	$C_{19}H_{34}O_2$	294	(*Z*,*Z*)-9,12-Octadecadienoic acid methyl ester	
22	37.05	$C_{19}H_{36}O_2$	296	(*Z*)-9-Octadecenoic acid methyl ester	
23	37.80	$C_{21}H_{44}$	296	Heptadecane, 2, 6, 10, 15 tetramethyl	
24	38.16	$C_{20}H_{40}O_2$	312	Octadecanoic acid, 10-methyl, methyl ester	
25	38.69	$C_{23}H_{48}$	324	9-Hexyl-heptadecane	
26	39.05	$C_{20}H_{38}O_2$	310	Cyclopropaneoctanoic acid, 2-octyl, methyl ester	
27	40.06	$C_{26}H_{54}$	366	Heneicosane, 11-(1-ethylpropyl)	
28	40.99	$C_{21}H_{42}O_2$	326	Methyl-18-methylnonadecanoate	
29	41.63	$C_{35}H_{70}$	490	17-Pentatriacontene	
30	41.95	$C_{18}H_{35}NO$	281	(*Z*)-9-Octadecenamide,	
31	43.98	$C_{44}H_{90}$	618	Tetratetracontane	
32	44.23	$C_{23}H_{46}O_2$	354	Docosanoic acid methyl ester	
33	44.35	$C_8H_5NO_6$	211	1,2-Benzenedicarboxylic acid, 3-nitro	
34	45.23	$C_{37}H_{76}O$	536	1-Heptatriacotanol	
35	45.76	$C_{24}H_{48}O_2$	368	Tricosanoic acid, methyl ester	
36	47.22	$C_{25}H_{50}O_2$	382	Tetracosanoic acid, methyl ester	
37	47.65	$C_{22}H_{46}$	310	Decosane	
38	48.30	$C_{30}H_{50}$	410	2, 6, 10, 14, 18, 22-Tertacosahexaene, 2, 6, 10, 15, 19, 23-hexamethyl	
39	49.88	$C_{38}H_{76}O_2$	564	9-Octadecene, 1-[2-(octadecyloxy) ethoxy]	

Table 3 In-vitro cholesterol-reducing activity of the methanolic extract of *Ganoderma* spp.

	Ganoderma spp. CRA (%)			
	Incubation time (h)			
Concentration of extract	24 h	48 h	72 h	96 h
1	35.1±1.51	54.2±0.95	72.6±1.85	83.4±1.93
2	44.3±0.20	66.0±0.60	85.8±0.90	91.2±1.31
3	60.1±0.70	74.8±0.26	87.8±0.30	94.9±1.30
4	61.5±0.78	75.2±0.26	88.5±0.50	99.0±1.05
5	63.5±1.06	77.3±0.60	90.5±1.05	100.0±0

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

The anti-rotavirus SA-11 activity of *Ganoderma* spp. extract

The cytotoxicity of the methanolic crude extract of *Ganoderma* spp. was investigated on MA 104 cells by the help of the MTT colorimetric assay. As shown in Table 4, *Ganoderma* spp. exerted toxic effects on MA 104 cells with CC_{50} of 650±0.80 µg/ml. This result indicated that this methanolic extract exhibited a promising anti-rotavirus activity with a TI of 9.3.

Table 4 Results of cytotoxicity and antiviral activity of the methanolic extract of *Ganoderma* spp. on MA 104 cells using the MTT method

Extract	CC_{50} (µg/ml)	IC_{50} (µg/ml)	TI
Ganoderma spp.	650±0.80	70±0.40	9.3

Each value represents the mean of three replicates (mean±SD). CC_{50}, 50% cytotoxic concentration; IC_{50}, 50% inhibitory concentration; TI, therapeutic index (CC_{50}/IC_{50}).

The anti-HCT116 human colon carcinoma activities of the methanolic crude extract

The cytotoxic effect of the methanolic crude extract was evaluated against HCT116 human colon carcinoma cell line. Results represented in Fig. 2 suggested that this extract had a promising cytotoxic effect, and that the sensitivity of the treated colon cells was concentration dependent. Treatment with *Ganoderma* spp. in concentration of 100 µg/ml resulted in a cytotoxicity of 84.03±0.93% whereas the positive control (cisplatin) in concentration of 50 µmol/l caused only 70.18±4.46% cytotoxicity.

Discussion

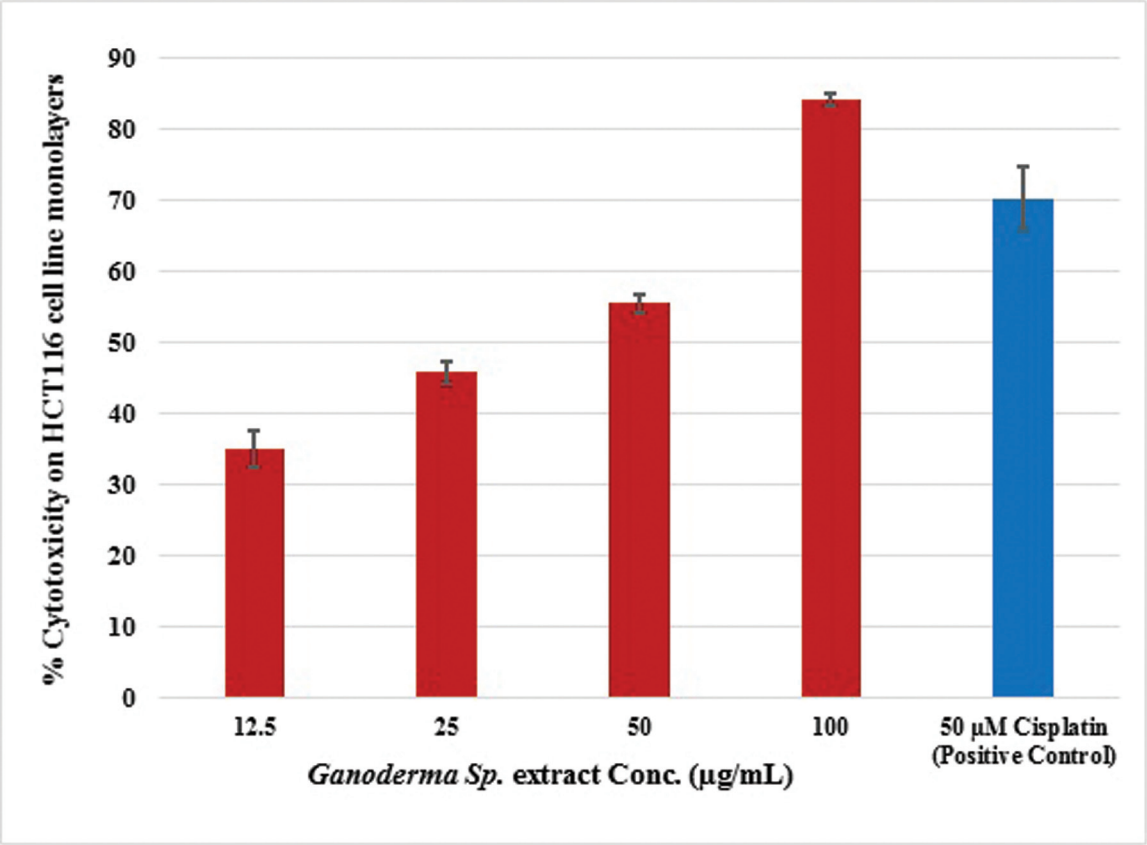
Species within the *Ganoderma* genus are proving to be promising sources of compounds with important biological activities. Many studies have previously reported numerous pharmacological properties of species within the *Ganoderma* genus [18,27–33].

The GC–MS analysis of the methanolic extract of *Ganoderma* spp. fruiting bodies resulted in the detection of 39 compounds. Majority of those compounds were alkenes, saturated and unsaturated fatty acids, which came in accordance with the GC–MS profile of some *Ganoderma* spp. that showed the presence of similar compounds [34,35].

The tested methanolic extract of *Ganoderma* spp. showed a remarkable cholesterol-reducing activity *in vitro*, indicating that *Ganoderma* spp. represents a promising source of biologically active compounds having hypocholesterolemic effects. Many studies have described the cholesterol-lowering activity of *Ganoderma* extracts and here we have quantified the impact in detail [36–38]. Previously, in the species *Ganoderma lucidum* the presence of some oxygenated lanosterol compounds were identified, and these work through inhibiting cholesterol synthesis in T9A4 hepatocytes to reduce total cholesterol and high-density lipoprotein % in tested hamsters to 9.8 and 11.2%, respectively [37]. However, α-glucans and β-glucans have also been nominated as compounds responsible for the cholesterol-lowering behavior of *G. lucidum* in mice [38]. On the other hand, many reports have pointed out polyunsaturated fatty acids as food constituents that reduce serum cholesterol [39–41]. In the current study, many unsaturated fatty acids have been detected in the extract of *Ganoderma* spp., such as octadecadienoic acid and oleic acid which may contribute in the hypocholesterolemic activity exerted by the extract.

Replication in viruses includes many steps such as attachment of the virus to the host, penetration of the host cell, replication of the virus within the host cell, assembly, and departure of the virus from the infected cells. Targeting these various steps can be used in the evaluation of the antiviral activities of different compounds [42]. In the current study, the effect of the methanolic extract of *Ganoderma* spp. on the attachment and penetration steps was investigated. As shown in Table 3, treatment with this extract resulted in an *in vitro* anti-RV SA-11 activity of TI 11, which indicated the capability of this extract to

Figure 2



Cytotoxicity % of *Ganoderma* spp. methanolic extracts on HCT116 cell line monolayers. Error bars represent the SD of three independent experiments.

attach to viral capsids, and hence stopping them from binding to cell receptors. Therefore, penetration and entry processes into host cells were prevented. Different compounds were previously identified from *Ganoderma applanatum* extracts and were nominated as antiviral agents [43].A promising *in-vitro* anti-human colon cancer activity was observed from treatment with *Ganoderma* spp. extract. This may be due to the presence of many unsaturated fatty acids such as oleic acid and octadecadienoic acid. Unsaturated fatty acids such as oleic acid have been nominated for their anticancer activities [44–46]. The mechanism of this action includes activating GPR40 and inducing oxidant stress and mitochondrial dysfunction in cancer cell lines [44]. It was also reported that free fatty acids can selectively inhibit the growth of tumor cells [47]. Moreover, a study conducted on the fatty acids from *G. lucidum* spores had proven its ability to inhibit tumor cell proliferation [46]. Octadecenes was also detected in the extract of *Ganoderma* spp. and it was reported for its anticancer activities [35,48]. On the other hand, reports for the anticancer activities of *Ganoderma* extracts explained this effect by the presence of many compounds such as applanoxidic acid C, D, F, G; nujiangexanthone B; heptemerone D; trichiol C; camphoratin E, xylariacin B, sphaeropsidin D, 7-methoxy-2,3,6-trimethylchromone, applanatumin A, and berkedrimane B [43,49–51].

Conclusion

Exploring the miraculous mushroom, *Ganoderma*, for biological activities is always resulting in promising outcomes. Results of this study highlighted the GC–MS analysis, in addition to the promising *in-vitro* capabilities of the methanolic crude extract of a Japanese *Ganoderma* spp. fruiting bodies. This extract exhibited hypocholesterolemic, anti-rotavirus, and a promising anti-human colon carcinoma activities. Investigating *Ganoderma* extracts and studying their potential therapeutic effects may contribute in the future in the identification of alternatives to the currently used drugs.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Rodriguez RJ, Redman RS, Henson JM. The role of fungal symbioses in the adaptation of plants to high stress environments. Mitig Adapt Strat Global Change 2004; 9:261–272.

2 World health organization cardiovascular-diseases-(cvds) fact sheet; 2017. Available at: http://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds). [Accessed on 2018 Nov 25]

3 Shimada Y, Yamakawa A, Morita T, Sugiyama K. Effects of dietary eritadenine on the liver microsomal 1, 6-desaturase activity and its mRNA in rats. Biosci Biotechnol Biochem 2003; 67:1258–1266.

4 Thompson PD, Panza G, Zaleski A, Taylor B. Statin-associated side effects. J Am Coll Cardiol 2016; 67:2395–2410.

5 Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 2003; 9:565–572.

6 Gray J, Vesikari T, Van Damme P, Giaquinto C, Mrukowicz J, Guarino A, *et al.* Rotavirus. J Pediatr Gastroenterol Nutr 2008; 2:S24–S31.

7 Rotavirus vaccines. WHO position paper – January 2013. J Wkly Epidemiol Rec 2013; 88:49–64.

8 Kim HH, Kwon HJ, Ryu YB, Chang JS, Cho KO, Hosmillo MD, *et al.* Antiviral activity of Alpinia katsumadai extracts against rota-viruses. Res Vet Sci 2012; 92:320–323.

9 World health organization cancer fact sheet; 2018. Available at: http://www.who.int/news-room/fact-sheets/detail/cancer. [Accessed on 2018 Nov 25]

10 Jones K. Reishi: ancient herb for modern times. Issaquah, WA: Sylvan Press. 1990. p. 6.

11 Ishikawa Y, Marimoto K, Hamasaki T. Flavoglaucin, a metabolite of *Eurotium chavalieri*, its oxidation and synergism with tocopherol. J Am Oil Chem Soc 1984; 61:1864–1868.

12 Komoda Y, Shimizu M, Sonoda Y, Sato Y. Ganoderic acid and its derivatives as Cholesterol synthesis inhibitors. Chem Pharm Bull (Tokyo) 1989; 37:531–533.

13 Ikekawa T. Beneficial effects of edible and medicinal mushrooms on health care. Int J Med Mushrooms 2001; 3:291–298.

14 Lindequist U, Niedermeyer TH, Jülich WD. The pharmacological potential of mushrooms. Evid Based Complement Alternat Med 2005; 2:285–299.

15 Barros L, Cruz T, Baptista P, Estevinho LM, Ferreira IC. Wild and commercial mushrooms as source of nutrients and nutraceuticals. Food Chem Toxicol 2008; 46:2742–2747.

16 Blagodatski A, Yatsunskaya M, Mikhailova V, Tiasto V, Kagansky A, Katanaev VL. Medicinal mushrooms as an attractive new source of natural compounds for future cancer therapy. Oncotarget 2018; 9:29259–29274.

17 Kirk PM, Cannon PF, Minter DW, Stalpers JA. Dictionary of the fungi. 10th ed. Wallingford: CABI. 2008. p. 272.

18 Paterson RR. Ganoderma: a therapeutic fungal biofactory. Phytochemistry 2006; 67:1985–2001.

19 Kendrick B. The fifth kingdom. Waterloo: Mycologue Publication; 1985.

20 Phillips R. Mushrooms: a comprehensiveguide to mushroom, identification. UK: Pan Macmillan; 2013

21 Xiao Z, Storms R, Tsang A. A quantitative starch–iodine method for measuring alpha-amylase and glucoamylase activities. Anal Biochem 2006; 351:146–148.

22 Pan D, Zhang D. Screening of cholesterol-reducing lactic acid bacteria and its activity in cholesterol-reducing. Food Sci 2005; 26:233–237.

23 Shaheen M, Borsanyiova M, Mostafa S, Chawla-Sarkar M, Bopegamage S, El-Esnawy N. In vitro effect of *Dodonaea viscosa* extracts on the replication of coxackievirus B3 (Nancy) and rotavirus (SA-11). J Microbiol Antimicrob Agents 2015; 1:47–54.

24 Finney DJ. Statistical method in biological assay. 3rd ed. New York, NY: Macmillan Publishing Co. Inc; 1978. 394–398

25 Nabil BS, Zyed R, Mohamed AL, Souad S, Mahjoub A. Assessment of the cytotoxic effect and in vitro evaluation of the anti-enteroviral activities of plants rich in flavonoids. J Appl Pharmaceut Sci 2:74–78.

26 Yang TT, Sinai P, Kain SR. An acid phosphatase assay for quantifying the growth of adherent and nonadherent cells. Anal Biochem 2012; 241:103–108.

27 Chang S, Buswell J. *Ganoderma lucidum* (Curt.: Fr.) P. Karst. (Aphyllophoromycetideae): a mushrooming medicinal mushroom. Int J Med Mushrooms 1999; 1:139–146.

28 Shiao MS. Natural products of the medicinal fungus *Ganoderma lucidum*: occurrence, biological activities, and pharmacological functions. Chem Rec 2003; 3:172–180.

29 Boh B, Berovic M, Zhang J, Zhi-Bin L. *Ganoderma lucidum* and its pharmaceutically active compounds. Biotechnol Annu Rev 2007; 13:265–301.

30 Mahajna J, Dotan N, Zaidman BZ, Petrova RD, Wasser SP. Pharmacological values of medicinal mushrooms for prostate cancer therapy: the case of *Ganoderma lucidum*. Nutr Cancer 2008; 61:16–26.

31 Patel S, Goyal A. Recent developments in mushrooms as anti-cancer therapeutics: a review. 3 Biotech 2012; 2:1–15.

32 Hapuarachchi KK, Cheng CR, Wen TC, Jeewon R, Kakumyan P. Mycosphere essays 20: therapeutic potential of Ganoderma species: Insights into its use as traditional medicine. Mycosphere 2017; 8:1653–1694.

33 Hapuarachchi KK, Elkhateeb WA, Karunarathna SC, Cheng CR, Bandara AR, Kakumyan P, *et al.* Current status of global Ganoderma cultivation, products, industry and market. Mycosphere 9:1025–1052.

34 Chen T, Wu J, Xu J, Li Y. Component analysis of fatty acids in spore lipid of Ganoderma lucidum (Reishi). J Fungal Res 2005; 3:35–38.

35 Orole OO. GC-MS evaluation, phytochemical and antinutritional screening of *Ganoderma lucidum*. J Adv Biol Biotechnol 2016; 5:1–10.

36 Kabir Y, Kimura S, Tamura T. Dietary effect of *Ganoderma lucidum* mushroom on blood pressure and lipid levels in spontaneously hypertensive rats (SHR). J Nutr Sci Vitaminol (Tokyo) 1988; 34:433–438.

37 Berger A, Rein D, Kratky E, Monnard I, Hajjaj H, Meirim I, *et al.* Cholesterol-lowering properties of *Ganoderma lucidum* in vitro, ex vivo, and in hamsters and minipigs. Lipids Health Dis 2004; 3:2.

38 Meneses ME, Martínez-Carrera D, Torres N, Sánchez-Tapia M, Aguilar-López M, Morales P, *et al.* Hypocholesterolemic properties and prebiotic effects of Mexican *Ganoderma lucidum* in C57BL/6 Mice. PLoS One 2016; 11:e0159631.

39 Hashimoto M, Shinozuka K, Hossain MS, Kwon YM, Tanabe Y, Kunitomo M, Masumura S. Antihypertensive effects of all-cis-5,8,11,14,17-icosapentaenoate of aged rats is associated with an increase in the release of ATP from caudal artery. J Vasc Res 1998; 35:55–62.

40 Gamoh S, Hashimoto M, Hossain M, Sugioka K, Hata N, Misawa Y, Masumura S. Chronic administration of docosahexaenoic acid improves reference memory-related ability in young rats. Neuroscience 1999; 129:70–76.

41 Hossain MS, Alam N, Amin SR, Basunia MA, Rahman A. Essential fatty acid contents of *Pleurotus ostreatus*, *Ganoderma lucidum* and *Agaricus bisporus*. Bangladesh J Mushroom 2007; 1:1–7.

42 Estes MK, Kapikian AZ. Rotaviruses. In: Knipe DM, Griffin DE, Lamb RA, Straus SE, Howley PM, Mar-tin MA, Roizman B, editors. Fields virology. 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2007. pp. 1917–1974.

43 Elkhateeb WA, Zaghlol GM, El-Garawani IM, Ahmed EF, Rateb ME, Moneim AE. *Ganoderma applanatum* secondary metabolites induced apoptosis through different pathways: *in vivo* and in vitro anticancer studies. Biom Pharmacother 2018; 101:264–277.

44 Yonezawa T, Katoh K, Obara Y. Existence of GPR40 functioning in a human breast cancer cell line, MCF-7. Biochem Biophys Res Commun 2004; 314:805–809.

45 Carrillo C, Cavia MD, Alonso-Torre SR. Oleic acid inhibits store-operated calcium entry in human colorectal adenocarcinoma cells. Eur J Nutr 2011; 51:677–684.

46 Lv GP, Zhao J, Duan JA, Tang YP, Li SP. Comparison of sterols and fatty acids in two species of Ganoderma. Chem Cent J 2012; 6:10.

47 Zhu YP, Su ZW, Li CH. Growth-inhibition effects of oleic acid, linoleic acid, and their methyl esters on transplanted tumors in mice. J Natl Cancer Inst 1989; 81:1302–1306.

48 Lee SH, Chang KS, Su MS, Huang YS, Jang HD. Effects of some Chinese medicinal plant extracts on five different fungi. Food Control 2007; 18:1547–1554.

49 Chairul SM, Hayashi Y. Lanostanoid triterpenes from *Ganoderma applanatum*. Phytochemistry 1994; 35:1305–1308.

50 Leon F, Valencia M, Rivera A, Nieto I, Quintana J, Estevez F, Bermejo J. Novel cytostatic lanostanoid triterpenes from *Ganoderma australe*. Helv Chim Acta 2003; 86:3088–3095.

51 Yuen JW, Gohel MD. Anticancer effects of Ganoderma lucidum: a review of scientific evidence. Nutr Cancer 2005; 53:11–17.