Insights into the in-vitro hypocholesterolemic, antioxidant, antirotavirus, and anticolon cancer activities of the methanolic extracts of a Japanese lichen, *Candelariella vitellina*, and a Japanese mushroom, *Ganoderma applanatum*

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Background and objective
Medicinal mushrooms, as well as lichens are potential sources of bioactive compounds against many lethal diseases. Therefore, in-vitro evaluations of the biological activities of the 80% methanolic extracts of two Japanese originated samples, namely, the mushroom *Ganoderma applanatum*, and the lichen *Candelariella vitellina* have been performed.
Materials and methods
Extraction of *C. vitellina* and *G. applanatum* was conducted using 80% methanol. The resulted extracts were examined for their in-vitro hypocholesterolemic and antirotavirus activities, as well as antihuman colon cancer action.
Results and conclusion
It was revealed that both extracts have potent hypocholesterolemic effects. *G. applanatum* in concentration of 24 mg/ml could successfully reduce the cholesterol concentration to 100±0% after 96 h of incubation at room temperature, whereas reaching this degree of reduction in cholesterol concentration required using 32 mg/ml of *C. vitellina* extract. On the contrary, the extract of *C. vitellina* at concentration of 1.0 mg/ml exhibited a stronger DPPH scavenging activity (99.5±0.166%), in comparison with 66.24±0.43% in case of using the same concentration of the *G. applanatum* extract. *C. vitellina* extract showed antirotavirus in a therapeutic index of 11, whereas *G. applanatum* extract recorded 3.4 only. The antihuman colon cancer study elucidated that both extracts have a moderate activity toward HCT116 human colon carcinoma. *C. vitellina* extract achieved a cytotoxicity of 57.90±4.4%, whereas the extract of *G. applanatum* displayed only 43.10±0.9%. Studies on *G. applanatum* and *C. vitellina* introduce them as promising sources of biologically potent compounds that can be candidates for use in treatment of many diseases after further studies.

Keywords:
biological activity, *Candelariella vitellina*, crude extract, *Ganoderma applanatum*, HCT116 human colon carcinoma cell line, hypocholesterolemic, rotavirus SA-11

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Introduction

Pharmaceutical and medicinal therapy advancement is rapid, and there are many success stories where new treatments have been developed and some infectious diseases have largely been eradicated [1–3]. Despite these successes, there are many life-threatening diseases that have not yet been mitigated or are advancing in importance, such as those associated with changes in lifestyle and population demographics. An example of this is hypercholesterolemia, a disease whose etiological origin has a multifactorial pattern, combining both genetic and environmental factors. As a critical risk factor for cardiovascular diseases (CVD), hypercholesterolemia is implicated in up to one-third of global human mortalities [4]. Here, managing the abnormal increase in cholesterol level can be used to prevent some CVD. Another example of a serious preventable disease is that caused by the rotavirus, which belongs to the family Retroviridae. Rotavirus is a major viral pathogen that causes diarrhea in both infants and young children worldwide, mostly in developing countries [5,6]. Yearly, more than 450 000 children younger than 5 years old die owing to rotavirus-induced diarrhea [7]. Although a rotavirus-specific vaccine exists, it has only been introduced by 96 countries worldwide (see: http://rotacouncil.org/vaccine-introduction/global-introduction-status/), and effective medication to completely control diarrhea

caused by Rotavirus has not yet been developed [8]. Finally, human colon cancer is a well-known disease being recognized as the third most common cancer and the fourth most common cause of cancer-related mortalities, worldwide [9–11]. Unfortunately, survival rates and prognosis for this type of cancer are often poor, and much work is needs to be done to enhance the detection and effectiveness of treatment of this disease.

The presence of these serious diseases and the currently available treatment options promote a need for detection of potent therapeutic compounds. The detection of such novel compounds may result in the development of promising alternatives or mutual prodrugs for currently used pharmaceutical options. In the current study, the activities of the methanolic crude extract of two samples collected from the Japanese mainland, namely, *Ganoderma applanatum* and *Candelariella vitellina*, were investigated through elucidating the effect on lowering cholesterol levels, cytotoxicity against human colon cancer, and antirotavirus capacity.

G. applanatum is a widely distributed wood-decaying mushroom, which has been used for centuries in Chinese traditional medicine and has previously been reported to possess potent antimicrobial, antifibrotic, and anticancer activities [12–14]. The second species, *C. vitellina*, investigated in this study is a yellow crustose lichen with a global prevalence [2,3]. Previously studied, the activities of metabolites of *C. vitellina* on a colorectal cancer cell line (Caco-2) resulted in an increase in apoptosis rate, which was associated with a p53 expression reduction besides elevation in the Bax/Bcl-2 ratio and caspase-3 mRNA level [2,3]. Moreover, *in vivo* treatment of a solid Ehrlich carcinoma with *C. vitellina* resulted in reduction in the tumor volume, which was dependent on the expression of p53 (data under publication), indicating that *C. vitellina* could represent a promising source of compounds with therapeutic potential.

Materials and methods

Collection and identification of samples

The *G. applanatum* specimen used in this study was collected from the trunk of a decaying Japanese cherry (Sakura) tree (*Prunus serrulata*) in Hakozaki, Fukuoka, Japan, and was identified by the Mycological Society of Japan as *G. applanatum*. All experiments were conducted *in vitro* and do not require ethics committee approval. The lichen used in this study was collected from the bark of a Japanese cherry (Sakura) tree (*Prunus serrulata*) in Hakozaki, Fukuoka, Japan, and it was identified as *Candelariella vitellina* by the Lichenological Society of Japan.

Extraction of the metabolites from mushroom

Approximately 250 g of fresh *G. applanatum* fruiting bodies was 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. Similarly, *C. vitellina* was subjected to the same 80% methanol extraction process. The total extract was evaporated and concentrated at 37°C using a rotary evaporator. Obtained extracts were stored at 4°C in a clean closed container until further use.

In-vitro cholesterol reduction assay

Overall, 0.4 g of the methanolic extracts of *G. applanatum* and *C. vitellina* was dissolved separately in 5 ml of distilled water, and 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. Moreover, 4 ml of distilled water supplemented with 1 ml of soluble cholesterol was used as a control. Finally, the percentage of cholesterol reducing activity was calculated as reported previously [15] as follows:,

$$\text{Cholesterol reducing activity(CRA\%)} = \left[(A_0A)/A_0\right] \times 100$$

Table 1 Preparation of different concentrations of extracts mixture for cholesterol-reducing activity assay

Extract concentration number	Added of extract (ml)	Added of distilled water (ml)	Added of cholesterol solution (ml)
1	0.25 (0.02 g)	3.75	1
2	0.5 (0.04 g)	3.5	1
3	1.0 (0.08 g)	3.0	1
4	1.5 (0.12 g)	2.5	1
5	2.0 (0.16 g)	2.0	1

where A0 is absorbance of the control (500 nm), and A is absorbance of the sample (500 nm). All tests were carried out in triplicate.

Antioxidant potential of extracts (DPPH assay)

The free radical scavenging activity using the 1.1-diphenyl-2-picryl-hydrazil (DPPH) reagent was determined as described previously [16]. In brief, different concentrations of *G. applanatum* and *C. vitellina* methanolic extract (10, 50, 100, and 500 µg/ml) were added separately to 1.0 ml of freshly prepared methanolic DPPH solution (20 µg/ml) followed by stirring. The decolorizing process was recorded after 30 min of reaction in darkness at 517 nm and compared with a blank control. The scavenging activity was evaluated spectrophotometrically using nanodrop (Thermo Fisher Scientific, South Carolina, USA, NanoDrop One Microvolume UV-Vis spectrophotometer) as described by Velazquez *et al.* [17]. A blank sample containing the same amount of solvent and DPPH solution was prepared and measured daily (A_{blank}). Different concentrations of ascorbic acid as a reference drug were used as positive controls. The radical scavenging activity was calculated from the following equation:

$$\text{Percentage of radical scavenging activity} = (A_{blank}A_{sample})/A_{blank} \times 100$$

where A_{sample} is the absorbance values of the test solution and A_{blank} is the sample containing the same amount of solvent and DPPH solution, which was prepared and measured daily. All tests were carried out in triplicate.

Antiviral activity of extracts 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 simian rotavirus SA-11 strains. 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 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.* [18]. The viral titers were calculated as $TCID_{50}$/0.1 ml (50% tissue culture infectious doses/0.1 ml) according to Spearman Kärber formula [19]. RV SA-11 stock was kept in small aliquots at −80°C until use.

Cytotoxicity assay

Different concentrations from each 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 extracts were examined onto MA 104 by using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [20]. In brief, 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 each extract (each dilution in triplicate). Cell control was included using only medium. The treated or non-treated cells were incubated for two days at 37°C in a 5% CO_2 incubator while checking the cell morphology under inverted microscope daily. After 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, 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 and T refer to the optical densities of cell control and treated cells, respectively.

Antiviral activity of extracts on RV SA-11 by MTT method

MA 104 at concentration of 5×10^4 cells/well was cultured for 24 h in CO_2 incubator at 37°C in 96-well microtiter plates. After removing the culture media, three non-toxic concentrations of each extract were tested against viral infections. Then 50 µl of 10^6 tissue culture infectious dose ($TCID_{50}$) 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. $TCID_{50}$ is defined as the concentration at which 50% of the cells are infected when a well plate upon which cells have been cultured is inoculated with a diluted solution of viral fluid.

After 1 h in CO_2 incubator, the mixed solution was removed. The cell lines were washed two times with culture medium, then 200 µl of 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

medium, were included in the assay. All plates were incubated for 3 days at 37°C in CO_2 incubator, and the cytopathic effect of the virus was monitored daily then measured by the MTT as described before. The percentage 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 ratio CC_{50} over IC_{50}.

Effect of crude extracts on HCT116 human colon carcinoma cell lines

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 5 A medium supplemented with 10% fetal bovine serum.

Cytotoxicity assay

The acid phosphatase assay was used to assess cytotoxicity according to the method described by Yang *et al.* [21]. Overall, 1×10^4 cells were seeded per well in 96-well plates, left to attach overnight, and then treated with samples for three days. For one plate, a substrate solution was prepared where 20 mg tablet of pNPP (catalog 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. Thereafter, 100 µl of pNPP substrate solution was added per well, and then plates were incubated for 4 h at 37°C. Then, 10 µl of 1 N sodium hydroxide stop solution was added 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 µmol/l cisplatin was used as positive control. Samples were tested at serial dilutions with final concentration of 400, 200, 100, 50, 25, 12.5, and 6.25 µg/ml. Percent cytotoxicity was calculated by the following formula:

$$[1-(D/S)]\times100$$

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

Results

The hypocholesterolemic activities of the crude extracts

Results in Table 2 illustrate in-vitro reduction of cholesterol by the methanolic extracts of *G. applanatum*, and *C. vitellina*. The results indicated that the cholesterol reduction activity increases proportionally with the increase in concentration of the extract, and elongation of incubation time. Both extracts exhibited high cholesterol reduction activity *in vitro* with varying degrees, ranging from 36.1±0.2 to 52.0±1.1% after 24 h, from 43.5±0.5 to 75.1±0.5% after 48 h, from 51.4±0.4 to 94.9±1.0% after 72 h, and from 60.8±0.6 to 100±0% after 96 h in case of *C. vitellina* extract. The highest cholesterol-reducing activity was achieved after 96 h of incubation at room temperature using concentration 5, which is equivalent to using 32 mg/ml of the *C. vitellina* methanolic crude extract. On the contrary, using the extract of *G. applanatum* resulted in cholesterol lowering activity, ranging from 54.1±1.0 to 89.2±0.8% after 24 h, from 63.1±0.6 to 92.7±0.9% after 48 h, from 72.6±1.4 to 96.3±0.9% after 72 h, and from 90.9±1.0 to 100±0% after 96 h. The highest cholesterol-reducing activity was achieved after 96 h of incubation at room temperature using concentration 4, which is equivalent to using 24 mg/ml of the *G. applanatum* methanolic crude extract.

The antioxidant DPPH free radical scavenging effect of the crude extracts

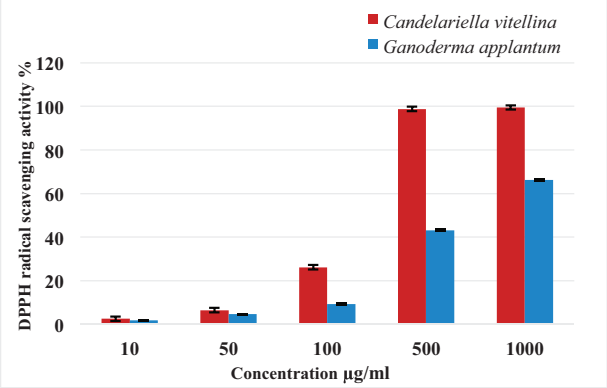
The antioxidant effects of the methanolic extracts of *C. vitellina* and *G. applanatum* were measured in terms of hydrogen-donating or radical scavenging capability, using the stable DPPH as reagent. The results illustrated in Fig. 1 revealed that those extracts exhibited significant concentration-dependent inhibition of DPPH activity. The data showed the superiority of the antioxidant ability of *C. vitellina*, which showed about 99.50±0.166% at concentration 1.0 mg/ml in comparison with 66.24±0.4.23 in case of *G. applanatum* at the same concentration. By decreasing the concentration to 0.1 mg/ml, the activities were decreased to 2.50±0.045% and 1.74 ±0.015 for *C. vitellina* and *G. applanatum*, respectively.

Table 2 In-vitro hypocholesterolemic activities of the methanolic extracts of *Candelariella vitellina* and *Ganoderma applanatum*

Concentration of extracts	*C. Vitellina* CRA (%)				*G. Applanatum* CRA (%)			
	Incubation time (h)							
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
1	36.1±0.2	43.5±0.5	51.4±0.4	60.8±0.5	54.1±1.0	63.1±0.6	72.6±1.4	90.9±1.0
2	38.5±0.8	59.1±0.4	79.7±0.5	83.4±0.7	64.2±1.5	70.0±0.3	77.0±1.2	93.9±2.0
3	41.9±0.6	66.3±0.8	87.2±1.0	94.3±1.6	67.2±0.3	76.8±1.0	85.8±0.3	98.6±0.4
4	50.7±0.7	71.4±1.1	91.2±1.4	97.3±1.0	81.1±1.4	86.8±1.0	92.2±0.6	100.0±0
5	52.0±1.1	75.1±0.5	94.9±1.0	100.0±0	89.2±0.8	92.7±0.9	96.3±0.9	100.0±0

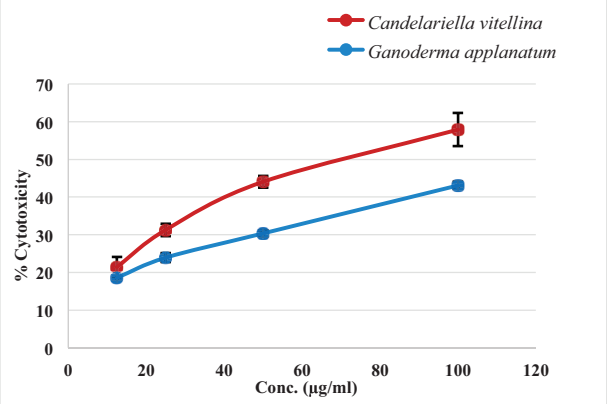
Each value represents the mean of three replicates (mean±SD).

Figure 1



DPPH radical scavenging activity % of the methanolic extracts of *Candelariella vitellina* and *Ganoderma applanatum* at different concentrations. Error bars represent the SD of three independent experiments.

Figure 2



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

Table 3 Results of cytotoxicity and antiviral activities of the methanolic extracts of *Candelariella vitellina* and *Ganoderma applanatum* on MA104 cells determined by MTT method

Extracts	Antiviral activity of extracts against RV SA-11		
	CC_{50} (µg/ml)[a]	IC_{50} (µg/ml)[b]	TI[c]
Candelariella vitellina	800±0.7	70±0.3	11
Ganoderma applanatum	1604.8±0.3	472±0.5	3.4

Each value represents the mean of three replicates (mean±SD).
[a]50% cytotoxic concentration. [b]50% inhibitory concentration.
[c]Therapeutic index (CC50/IC50).

The anti-rotavirus SA-11 activities of extracts

Crude extracts were investigated for their cytotoxic effects on MA 104 cells using MTT colorimetric assay. *C. vitellina* and *G. applanatum* showed toxic effects on MA 104 cells with CC50 of 800±0.7 and 1604.8±0.3 µg/ml, respectively (Table 3). These results indicated that both methanolic extracts exert a promising anti-rotavirus activity. The crude extract of *C. vitellina* exhibited a TI of 11, whereas *G. applanatum* extract displayed a TI of 3.4.

The anti-HCT116 human colon carcinoma activities of methanolic crude extracts

The cytotoxic effect of both crude extracts was evaluated toward HCT116 human colon carcinoma cell line. Results represented in Fig. 2 depicted that these extracts had a moderate cytotoxic effect, and that the sensitivity of the treated colon cells was concentration dependent. An obvious cytotoxic effect was obtained by treatment with *C. vitellina* extract which exhibited cytotoxicity of 57.90±4.4%, whereas *G. applanatum* exerted a cytotoxicity of 43.10 ±0.9%.

Discussion

The clock is ticking in our battle against a number of diseases that are implicated in increasing rates of mortality among infants, children, and adults. The development of new therapeutic compounds or supporting compounds to aid the currently used suite of drugs is a practical solution to face such serious diseases. The aim of this study was to explore, highlight, and compare the in-vitro hypocholesterolemic, antioxidant, antirotavirus, and anticolon cancer activities of the crude extracts of the lichen, *C. vitellina*, with those of the mushroom *G. applanatum*. LC-HRMS chemical analysis of the methanolic extract of *G. applanatum* used in this study has previously been reported [14]. Moreover, LC-HRMS analysis was conducted on the methanolic extract of *C. vitellina* [22].

The in-vitro cholesterol-reduction analysis revealed that the methanolic extract of *G. applanatum* not only decreased cholesterol concentration, but it did so to a higher degree and at lower concentration levels than extracts of *C. vitellina* (Table 2). A concentration as low as 24 mg/ml of *G. applanatum* methanolic extract showed superiority over that of *C. vitellina*. The hypocholesterolemic properties of some fungi have previously been widely reported, with the presence of lovastatins being cited as causative factor in a number of cases [20,23–29]. However, statins were not on the list of identified chemical compounds in *G. applanatum* extract [14], meaning that the therapeutic compounds found in this fungus is potent and different.

Many serious diseases such as cancer, atherosclerosis, CVDs, Alzheimer's, and diabetes and some cases of

drug-induced toxicity can be caused by the accumulation of excess free radicals, which are produced as part of normal cell functioning [30,31]. Therefore, exploring novel antioxidants is of great importance. Results of DPPH radical scavenging activity in the current study (Fig. 1) revealed that the extract of *C. vitellina* exhibited a superior antioxidant activity (99.50±0.166%) in comparison with that exerted by *G. applanatum* (66.2±0.42%). Many studies have reported the antioxidant effect of lichens extracts [32–34], and generally this antioxidant activity was caused by the presence of various phenolic and flavonoid compounds [32,35,36]. On the contrary, the antioxidant activity of *G. applanatum* may be explained by the presence of other compounds known for their antioxidant activity such as the polyketide (Daldinin F), an isoflavonoid (7-o-Glucosyl-daidzein), and the terpene, applanoxidic acid E, among other terpenes that are famous for their antioxidant activity [14].

The viral replication cycle consists of various steps starting with attachment, penetration, replication of viral proteins and genetic materials, assembly, and ending with the escape of the virus from infected cells. These different steps can be individually targeted to evaluate compounds for their activity against rotavirus SA-11 [37].

In this study, we elucidated the effect of tested extracts on both the attachment and penetration steps. As shown in Table 3, the higher antiviral behavior resulted from extract of *C. vitellina* against RV SA-11 infection with TI=11, whereas *G. applanatum* extract exhibited TI=3.4 against virus infection. These results indicated that the extracts may have the ability to attach to viral capsids preventing them from binding to cell receptors, and hence preventing their penetrations and entry into host cells. Many compounds having antiviral activities have been identified previously from the 80% methanolic extract of *G. applanatum* (the same sample used in the current study), such as the alkaloid (8-demethoxyhostasine) [14]. In terms of lichens, the antiviral activities were described several times previously [38]. Polysaccharide fraction extracted from *Parmelia perlata* displayed an antiyellow fever virus action [39,40]. Moreover, the compound atranorin was responsible for the antihepatitis C activity of the lichen *Stereocaulon evolutum* through inhibiting viral entry [41].

The in-vitro anticancer study using these crude extracts showed moderate cytotoxicity behavior against human colon carcinoma cells lines, which in the case of *G. applanatum* may be attributed to the presence of terpenes, which are famous for their antitumor activities such as applanoxidic acid C, D, F, and G [14], along with assortment of compounds carrying various cytotoxic and antifibrotic effects such as nujiangexanthone B, heptemerone D, trichiol C, camphoratin E, xylariacin B, sphaeropsidin D, 7-methoxy-2,3,6-trimethylchromone, applanatumin A, and berkedrimane B [14,42]. Interestingly, here we present *C. vitelline* as having greater cytotoxic activity than *G. applanatum*. This can be attributed to the richness of this *C. vitelline* sample with various cytotoxic, as well as anticancer compounds, which have been detected in this extract in a previous study, such as hericenone A, comazaphilone C, hormothamnione, arnottianamide, ovellin B, ganodermatriol, fomefficinic acid A, and ganoderol A and F [22]. Many other studies have shown anticancer activities of lichen species [43]. For example, Millot *et al.* [44] reported the potency of the acetone extracts of *Pleurosticta acetabulum* against human colon cancer cells HT29, exhibiting an IC_{50} of 6 μg/ml after 48 h of treatment. Furthermore, Ren *et al.* [45] presented the anti-cancer activities of acetone extract of *Lethariella zahlbruckneri* toward HT-29 human colon cancer cells, inhibiting HT-29 cell proliferation through inducing apoptosis, which could be mediated via two pathways. Consequently, there appears strong potential for identifying novel cytotoxic compounds in lichens.

Conclusion

Throughout this study, we have demonstrated potent hypocholesterolemic, antiviral, antioxidant, and cytotoxic effects of extracts from both the fungus *G. applanatum* and the lichen *C. vitellina*. Although there is some understanding as to how these extracts are exerting an effect, further work will be needed to isolate and purify compounds responsible for those biological activities. In summary, this study represents an important step toward identifying and isolating potentially novel compounds with significant therapeutic potential.

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Conflicts of interest

There are no conflicts of interest.

References

1 Demain AL, Zhang L. Natural products and drug discovery. Nat Product 2005; 13:3–29.

2 Gurnani N, Mehta D, Gupta M, Mehta B. Natural Products: source of potential drugs. African J Basic Appl Sci 2014; 6:171–186.

3 David B, Wolfender JL, Dias DA. The pharmaceutical industry and natural products: historical status and new trends. Phytochem Rev 2015; 14:299–315.

4 World Health Organization. Cardiovascular diseases (CVDS) fact sheet. Available at: http://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds). [Accessed 17 May 2017].

5 Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. Emerging Infect Disease 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 World Health Organization. Rotavirus Vaccines: WHO Position Paper, Weekly Epidemiological Record No. 5 88. Available at: http://www.who.int/wer/2013/wer8805.pdf. February 2013; p. 50 Geneva

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 Veterinary Sci 2012; 92:320–323.

9 Favoriti P, Carbone G, Greco M, Pirozzi F, Pirozzi R, Corcione F. Worldwide burden of colorectal cancer: a review. Updates Surg 2016; 68:7–11.

10 Arnold M, Sierra M, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut 2017; 66:683–691.

11 Sadeghi Gandomani H, Aghajani M, Mohammadian-Hafshejani A, Asgari Tarazoj A, Pouyesh V, Salehiniya H. Colorectal cancer in the world: incidence, mortality and risk factors. Biomed Res Ther 2017; 4:1656–1675.

12 Usui T, Iwasaki Y, Hayashi K, Mizuno T, Tanaka M, Shinkai K, Arakawa M. Antitumor activity of water-soluble β-D-glucan elaborated by *Ganoderma applanatum*. Agricult Biol Chem 1981; 45:323–326.

13 Usui T, Iwasaki Y, Mizuno T, Tanaka M, Shinkai K, Arakawa M. Isolation and characterization of antitumor active β-D-glucans from the fruit bodies of *Ganoderma applanatum*. Carbohydrate Res 1983; 115:273–280.

14 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. Biomed Pharmacother 2018; 101:264–277.

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

16 Brand-Williams W, Cuvelier M, Berset CL. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 1995; 28:25–30.

17 Velazquez E, Tournier H, De Buschiazzo P, Saavedra G, Schinella G. Antioxidant activity of Paraguayan plant extracts. Fitoterapia 2003; 74:91–97.

18 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.

19 Finney DJ. Statistical Method in Biological Assay. New York, NY: Macmillan Publishing Co. Inc; 1978. 394–398

20 Nabil B, Zyed R, Mohamed A, 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 Pharm Sci 2012; 2:74–78.

21 Yang TT, Sinai P, Kain SR. An acid phosphatase assay for quantifying the growth of adherent and nonadherent cells. Anal Biochem 1996; 241:103–108.

22 El-Garawani IM, Elkhateeb WA, Zaghlol GM, Almeer RS, Ahmed EF, Rateb ME, Moneim AE. Candelariella vitellinaextract triggers in vitro and in vivo cell death through induction of apoptosis: a novel anticancer agent. Food Chem Toxicol 2019; 127:110–119.

23 Kaneda T, Tokuda S. Effect of various mushroom preparations on cholesterol levels in rats. J Nutr 1966; 90:371–376.

24 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 1988; 34:433–438.

25 Cheung PC. The hypocholesterolemic effect of extracellular polysaccharide from the submerged fermentation of mushroom. Nutr Res 1996; 16:1953–1957.

26 Ozdín L, Galbavý Š. Dose-and time-dependent hypocholesterolemic effect of oyster mushroom (*Pleurotus ostreatus*) in rats. Nutrition 1998; 14:282–286.

27 Gunde-Cimerman N, Plemenitas A. Hypocholesterolemic activity of the genus *Pleurotus* (Jacq.: Fr.) *P. Kumm*. (Agaricales s. I., Basidiomycetes). Int J Med Mushrooms 2001; 3:A91.

28 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 Disease 2004; 3:2–13.

29 Meneses M, 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.

30 Hajhashemi V, Vaseghi G, Pourfarzam M, Abdollahi A. Are antioxidants helpful for disease prevention? Res Pharm Sci 2010; 5:1.

31 Leopold JA. Antioxidants and coronary artery disease: from pathophysiology to preventive therapy. Coronary Artery Dis 2015; 26:176–183.

32 Kosanić MA, Ranković BR. Lichens as possible sources of antioxidants. Pak J Pharm Sci 2011; 24:165–170.

33 Mitrović T, Stamenković S, Cvetković V, Tošić S, Stanković M, Radojević I, *et al.* Antioxidant, antimicrobial and antiproliferative activities of five lichen species. Int J Mol Sci 2011; 12:5428–5448.

34 Fernández-Moriano C, Gómez-Serranillos M, Crespo A. Antioxidant potential of lichen species and their secondary metabolites. A systematic review. Pharm Biol 2016; 54:1–17.

35 Kodama N, Komuta K, Nanba H. Effect of Maitake (*Grifola frondosa*) D-Fraction on the activation of NK cells in cancer patients. J Med Food 2003; 6:371–377.

36 Kosanić M, Ranković B. Antioxidant and antimicrobial properties of some lichens and their constituents. J Med Food 2011; 14:1624–1630.

37 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. 1917–1974

38 Karagoz A, Aslan A. Antiviral and cytotoxic activity of some lichen extracts. Biol Bratislava 2005; 60:281–286.

39 Esimone CO, Ofokansi KC, Adikwu MU, Ibezim EC, Abonyi DO, Odaibo GN, Olaleye DO. In vitro evaluation of the antiviral activity of extracts from the lichen *Parmelia perlata* (L.) Ach. Against three RNA viruses. J Infect Develop Countries 2007; 1:315–320.

40 Van Cuong T, Thoa N. Bioactive compounds from lichens as promising biomaterial for the treatment of influenza virus: a review. J Sci Res Rep 2018; 18:1–15.

41 Vu TH, Le Lamer AC, Lalli C, Boustie J, Samson M, Lohézic-Le Dévéhat F, Le Seyec J. Depsides: lichen metabolites active against hepatitis C virus. PLoS One 2015; 10:e0120405.

42 Luo Q, Di L, Dai WF, Lu Q, Yan Y, Yang Z, *et al.* Applanatumin A, a new dimeric meroterpenoid from *Ganoderma applanatum* that displays potent antifibrotic activity. Organic Lett 2015; 17:1110–1113.

43 Boustie J, Grube M. Lichens a promising source of bioactive secondary metabolites. Plant Genet Resour 2005; 3:273–287.

44 Millot M, Delebassée S, Liagre B, Vignaud L, Sol V, Mambu L. Screening of lichen extracts on HT-29 human colon-cancer cells. Planta Medica 2014; 80:P1N5.

45 Ren MR, Hur JS, Kim JY, Park KW, Park SC, Seong CN, *et al.* Anti-proliferative effects of *Lethariella zahlbruckneri* extracts in human HT-29 human colon cancer cells. Food Chem Toxicol 2009; 47:2157–2162.