DNA-binding activity and in vivo cytotoxicity of Ganoderma applanatum (Pers.) Pat. supercritical-CO$_2$ extracts

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Ganoderma applanatum (Pers.) Pat. is a perennial and abundant mushroom in Bukidnon Province, Philippines but its bioactivities have just been investigated lately. In this study, three supercritical-CO$_2$ (SC-CO$_2$) extracts (GA1, GA2 and GA3) from the local strain of G. applanatum (Pers.) Pat. were examined for their DNA-binding potential by means of biomolecular-chemical screening and in vivo cytotoxicity using brine shrimp lethality assay (BSLA). All extracts contained secondary metabolites that have DNA-binding activity towards salmon sperm DNA (Rf$_2$/Rf$_1$-ratio <1). For BSLA, the LC$_{50}$ (24 h) values ranged from 177.82 to 354.81 µg/ml, indicating that the extracts are biologically active but with no significant cytotoxicity. Reverse-phase HPLC and FT-IR spectroscopy were carried out to partially characterize the extracts. Results of this study showed that G. applanatum (Pers.) Pat. from Bukidnon Province, Philippines is a potential source of biologically active, non-toxic compounds with DNA-binding activity.

Key words: Ganoderma applanatum, DNA-binding activity, cytotoxicity, supercritical-CO$_2$.

INTRODUCTION

Mushrooms have been valued for generations as source of medicines or as food. For centuries, the medicinal use of mushrooms such as Ganoderma was recorded in China, Japan and Korea (Paterson, 2001). Of the approximately 10,000 known species of mushrooms, 2000 are safe for human health and about 300 of them possess medicinal properties (Wasser and Weis, 1999). Mushrooms can synthesize a dazzling array of biologically-active products which have no significant role in their primary physiological processes (Kidukuli et al., 2010). These are secondary metabolites that have often attracted interest because of their biological effect on other organisms. The strong activities of these bioactive compounds enable mushrooms to survive in their environments and allow them to out-compete their environmental competitors (Kidukuli et al., 2010). It is not surprising therefore that mushrooms contain secondary metabolites that may have economic and pharmaceutical values.

Ganoderma is a prolific producer of novel “mycochemicals” (Paterson, 2006). There are reports on its folkloric use for the treatment of various ailments (Rowan et al., 2002; Jeong et al., 2008). Phytochemical studies of Ganoderma applanatum (Pers.) Pat. showed that it contains triterpenoids, tannins, sterols, steroids, proteins and polysaccharides (Boh et al., 2000; de Silva et al., 2006; Chairul et al., 1991; Strigina et al., 1971). Previous studies demonstrated its antioxidant, antimicrobial, immunomodulating, aldose reductase inhibitors, nitric oxide synthase activators and anti-tumor properties (Barranco et al., 2010; Muhsin et al., 2011; Smania et al., 1999; Lee et al., 2005; Acharya et al., 2005; Jeong et al., 2008; Chairul et al., 1991). However,
the DNA-binding potential of its secondary metabolites have not been investigated. DNA-binding molecules proved that they can be effective anticancer, antibiotic and antiviral therapeutic agents (Gibson, 2002). In fact, over 60% of the clinical anticancer drugs introduced in 2002 are natural products or natural products derivatives, and most exert their effects by acting on DNA (Newman et al., 2003). It is therefore important to provide information on the DNA-binding activity of the secondary metabolites from G. applanatum (Pers.) Pat. for potential bio-prospecting.

In the Philippines, G. applanatum (Pers.) Pat. is abundant in the wild but is largely untapped due to the common notion that it contains toxic substances that might be deleterious to humans. It is also overshadowed by the influx of edible and cultivated mushroom species, thus scientific research on this basidiomycete species is scarce. The fact that the toxicity of a certain compound may suggest its potential as anti-tumor or anticancer, a study on the aspect of cytotoxicity is important. It is therefore imperative to assess the bioactivities of Philippine wild G. applanatum (Pers.) Pat. to tap this abundant potential source of bioactive secondary metabolites.

The aim of this study is to determine the DNA-binding potential and in vivo cytotoxic activity of the supercritical-CO$_2$ (SC-CO$_2$) extracts of G. applanatum (Pers.) Pat. from Bukidnon Province, Philippines by means of biomolecular-chemical screening (Maier et al., 1998) and brine shrimp lethality assay (BSLA), respectively. Furthermore, reverse-phase HPLC and FTIR spectroscopy were carried out to partially characterize the extracts.

MATERIALS AND METHODS

Mushroom samples

Fresh basidiocarps of G. applanatum (Pers.) Pat. were collected from the wild in the Bukidnon Province, Central Mindanao, Philippines. Samples were identified and authenticated through differentiating characteristics as described by Leonard (1998), and by comparing with voucher specimens of previous collections deposited in the Molecular Biology and Biotechnology Laboratory and at the Natural Science Museum, both at MSU-IIT.

Sample preparation and supercritical-CO$_2$ extraction

G. applanatum (Pers.) Pat. basidiocarps were cut into pieces (1 x 1 mm) and air-dried to achieve the <14% moisture content. The cut and dried fruit bodies were pulverized using a mechanical grinder. The pulverized fruit bodies were contained in the white cloth bag and later in the SC-CO$_2$ metal sample cartridge and submitted for SC-CO$_2$ extraction using a supercritical fluid extractor (Akico). After stabilization for 5 min, extraction was followed using three (3) pressure levels: 10, 20 and 30 MPa in 40°C in a flow rate of 0.5 ml/min, and the resulting extracts were labeled GA1, GA2 and GA3, respectively. The extracts were collected in 5 ml test tubes, sealed with laboratory film, wrapped with aluminum foil and stored in the refrigerator until use. These extracts are solvent-free.

Determination of DNA-binding activity

The biomolecular-chemical screening using two-dimensional thin layer chromatography (2D-TLC) protocol as reported and described by Maier et al. (1999a, 1999b) was adapted with slight modifications for determining the DNA-binding activity of the extracts. Homogenized salmon sperm DNA was purchased from Chemline Scientific Enterprises (11 mg/ml). DNA fragments are between 300 and 3000 base pairs in size. For the working solution, 181 µl of DNA sample was withdrawn from the stock. This was diluted with 819 µl of sterile distilled water to have a concentration of 2 mg/ml. Homogenized DNA was denatured by heating at 95°C for 10 min and cooling on ice immediately after. Sample was stored at -20°C until it was used in the biomolecular-chemical screening. For 2D-TLC, the TLC plates (TLC silica gel 60 F254 Merck) were cut into a 6.5 x 6.5 cm dimension. The extracts were spotted at one corner of the TLC plate with 1 cm distance from the base and the side of the plate. Two plates were prepared for each extract, the measuring plate and the reference plate. For the first development, GA2 and GA3 were run in a solvent system constituting chloroform : ethyl acetate : methanol (55:35:10) and GA1 was run in a solvent system constituting chloroform : methanol (95:5). These solvent systems were determined after several trials. Afterwards, the separations produced were viewed under UV light (254 nm). Interaction with DNA was analyzed in the second dimension (2D-TLC) in the solvent system constituting chloroform : methanol : glacial acetic acid (47.5:47.5:5). DNA was spotted in the measuring plate above the separations using non-heparinized capillary tube (5 µg DNA/cm) before the second chromatographic step. Detection of DNA-binding was performed by means of UV light at 254 nm. Changes in the Rf-values indicate an interaction between ligand and DNA and were expressed by the Rf$_2$/Rf$_1$-ratio, in which Rf$_1$ represents the Rf-value without DNA (reference plate), and Rf$_2$ represents the Rf-value with DNA (measuring plate). Rf$_2$/Rf$_1$ ratios decreased below 1 if DNA-binding occurs (Maier et al., 1999a). To eliminate the dilemma in classifying Rf$_2$/Rf$_1$ ratios that reached 1 if rounded off, the ratios that fell below 1 were categorized as either they had strong or weak affinity to DNA. Rf$_2$/Rf$_1$ ratios which are <0.85 are considered moderate to strong DNA-binders and the Rf$_2$/Rf$_1$ ratios which are >0.85 are considered weak DNA-binders (Maul et al., 1999).

Cytotoxic activity

Cytotoxic activity of the extracts was determined through the brine shrimp lethality assay. This assay was carried out according to the principle and protocols previously described by Meyer et al. (1982) and McLaughlin et al. (1998) with slight modifications. Brine shrimp eggs (Artemia salina Leach) were purchased from a local pet shop. The eggs were hatched in a small tank with two unequal-sized chambers filled with boiled, filtered sea water. One chamber of the tank was covered with aluminum foil and fully aerated. After 48 h incubation at room temperature and under illumination (18 W bulb), the resulting nauplii (larvae) were attracted to the other side of the tank with a light source and collected with a Pasteur pipette. Samples for test were prepared by initially dissolving 20 mg of extract in 2 ml dimethyl sulfoxide (DMSO) and further diluted with sea water to produce the required concentrations. Appropriate amounts (200, 20 or 2 µl for 1000, 100 and 10 µg/ml, respectively) were transferred to vials. Ten 24-h old nauplii were transferred to each sample vial, and boiled, filtered sea water was added to make 5 ml. Tests for each concentration were done in triplicate. A control experiment containing 200, 20 or 2 µl DMSO in 5 ml boiled, filtered sea water and ten nauplii was also performed in triplicate for each concentration. The vials were placed uncovered under 18 W fluorescent bulb for 24 h after which the survivors were counted and the percentage mortality at each vial and control was determined.
using the equation:

\[
\text{Mortality} (\%) = \frac{\text{(No. of dead nauplii/initial no. of live nauplii)}}{\times 100}
\]

Probit analysis by Finney (1971) was used to calculate the concentration at which lethality to brine shrimps represents 50% (LC50). Extracts with LC50 values <1000 ppm were considered biologically active and extracts with LC50 values <100 ppm (100 µg/ml) were considered to have significant cytotoxicity (Montanher et al., 2002; Gupta et al., 1996; Peteros and Uy, 2010).

High-performance liquid chromatography

The samples were subjected to reverse-phase HPLC (Perkin-Elmer) equipped with LC18 column (Wakopak) and spectrophotometric detector LC290. Absolute HPLC-grade methanol (Scharlau) was used as mobile phase. All samples were dissolved in 1 ml absolute HPLC-grade methanol (Scharlau) and filtered with disc filter before injecting (20 µl injection volume) into the HPLC apparatus. The flow rate was 1 ml/min and the wavelength of detection was 254 nm.

Fourier transform infra-red spectroscopy

Spectrum 100 Fourier-transform infrared (FT-IR) spectrometer (Perkin-Elmer) was used for the chemical elucidation of extracts. Each sample was put on the attenuated total reflectance (ATR) sample well and was scanned at room temperature with a range of 4000 to 550 cm\(^{-1}\). The resulting spectra were analyzed and functional groups were assigned to the functional group region, between 1300 and 4000 cm\(^{-1}\).

**RESULTS**

**DNA-binding activity**

The DNA-binding activity of *G. applanatum* (Pers.) Pat. was determined through biomolecular-chemical screening on a 2D-TLC format. Results (Table 1) revealed that the extracts contained secondary metabolites with DNA-binding activity as shown by their Rf2/Rf1-ratios which are below 1. The number of components separated by one-dimensional TLC varies, indicating that the three extracts contained different numbers of secondary metabolites. Of the three extracts, GA1 contained secondary metabolites that all have DNA-binding activity.

**In vivo cytotoxicity**

Results of cytotoxic activity through BSLA of SC-CO\(_2\) extracts of *G. applanatum* (Pers.) Pat. (% mortality at different concentrations and LC50 values at 24 h) are shown in Table 2. No mortality was observed in the lowest concentration (10 µg/ml). However, all extracts

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**Table 1. DNA-binding activity of the SC-CO\(_2\) extracts as revealed by two-dimensional thin layer chromatography.**

<table>
<thead>
<tr>
<th>Extract</th>
<th>Solvent system for first development (1D)</th>
<th>Solvent system for second development</th>
<th>No. of separated spots in the first development (1D)</th>
<th>1D Rf value w/o DNA (Rf1)</th>
<th>1D Rf value w/DNA (Rf2)</th>
<th>2D Rf values Rf2/Rf1 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA1</td>
<td>chloroform: methanol (95:5)</td>
<td>chloroform: methanol: glacial acetic acid (47.5:47.5:5)</td>
<td>3</td>
<td>0.24</td>
<td>0.28</td>
<td>0.24</td>
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<td></td>
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<td></td>
<td></td>
<td>0.56</td>
<td>0.42</td>
<td>0.32</td>
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<td></td>
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<td></td>
<td></td>
<td>0.80</td>
<td>0.94</td>
<td>0.90</td>
</tr>
<tr>
<td>GA2</td>
<td>chloroform: ethyl acetate:methanol (55:35:10)</td>
<td>chloroform: methanol: glacial acetic acid (47.5:47.5:5)</td>
<td>4</td>
<td>0.40</td>
<td>0.68</td>
<td>0.66</td>
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<td></td>
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<td></td>
<td></td>
<td>0.44</td>
<td>0.84</td>
<td>0.80</td>
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<td></td>
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<td></td>
<td></td>
<td>0.80</td>
<td>0.86</td>
<td>0.86</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.84</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>GA3</td>
<td>chloroform: ethyl acetate:methanol (55:35:10)</td>
<td>chloroform: methanol: glacial acetic acid (47.5:47.5:5)</td>
<td>3</td>
<td>0.40</td>
<td>0.66</td>
<td>0.60</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.58</td>
<td>0.78</td>
<td>0.74</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.84</td>
<td>0.88</td>
<td>0.88</td>
</tr>
</tbody>
</table>

**Table 2. Results of brine shrimp lethality assay on SC-CO\(_2\) extracts from *G. applanatum* (Pers.) Pat.**

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Mortality at different concentrations (%)</th>
<th>LC50, 24 h (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 µg/ml</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>GA1</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>GA2</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>GA3</td>
<td>0.00</td>
<td>13.33</td>
</tr>
<tr>
<td>Negative</td>
<td>0.00</td>
<td>0.00</td>
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</tbody>
</table>

**Mean of 3 replicates.**
showed 100% mortality to brine shrimp at 1000 µg/ml. LC$_{50}$ values ranged from 177.82 to 354.81 µg/ml, with GA3 having the lowest value, thus, the most potent. It means that it will take only 177.82 µg/ml of the extract to kill half of the total individuals of the test species. GA1 showed the highest LC$_{50}$ value (354.81 µg/ml). This result does not show significant toxicity towards the brine shrimp, which could be manifested by LC$_{50}$ more than 100 ppm. DMSO, which act as negative control showed no toxicity to brine shrimps (LC$_{50}$ = 0 µg/ml).

### Reverse-phase HPLC

Figure 1A, B and C shows the HPLC chromatograms of GA1, GA2 and GA3, respectively. All chromatograms show at least one dominant peak, suggesting the presence of a secondary metabolite in high concentration. The similarity of the retention time of the dominant peaks suggests that they share the same chemical property such as polarity. The absence of numerous peaks in the chromatograms also suggests that the SC-CO$_2$ extracts of *G. applanatum* (Pers.) Pat. are of high purity.

### FT-IR spectroscopy

The IR spectra of *G. applanatum* (Pers.) Pat. extracts GA1, GA2 and GA3 are shown in Figure 2. There were several main absorption bands (peaks) in the spectra of these mushroom extracts. The three spectra are observably almost similar. Although, the absorption bands differ in few cm$^{-1}$, they still fall within the baseline levels of respective functional groups. GA2 showed one band at 3418.32 cm$^{-1}$ which is absent in GA1 and GA3. This peak is due to the NH$_2$ stretch for amines or OH stretch for alcohols. The rest of the peaks are present in all extracts. The absorption bands at 2923.88, 2923.52 and 2923.87 cm$^{-1}$ in GA1, GA2 and GA3, respectively, correspond to C—H asymmetric stretching of the aliphatic CH$_2$ groups, which may also indicate the
presence of various amino acids. The absorption bands at 2854.21 cm\(^{-1}\) in GA1, 2853.03 cm\(^{-1}\) in GA2 and 2853.21 cm\(^{-1}\) in GA3 are due to the very strong symmetric stretch of aliphatic —CH\(_2\)—. These peaks are ubiquitous in organic compounds. The absorption bands at 1707 to 1741 cm\(^{-1}\) are assigned to carbonyl stretching (C=O) which may come from fatty acids and lipids or to C=O stretch for saturated aliphatic esters or carboxylic acid anhydrides. The bands 1458 to 1460 cm\(^{-1}\) are due to the asymmetric bending of aliphatic CH\(_3\). These bands together with the bands 2923 to 2925 cm\(^{-1}\) may both receive contributions from fatty acids and lipids. The presence of narrow peaks at 1372 to 1377 cm\(^{-1}\) was assigned to the moderate symmetric bending of aliphatic CH\(_3\). The peaks <1300 cm\(^{-1}\) were not assigned to functional groups, since this a fingerprint region. The IR spectra of the extracts differ in the sharpness of the peaks, indicating the differences in the concentration and purity of the chemical constituents present in the extracts. FT-IR spectra also indicate the presence of other functional groups such as amines, esters and carbonyl groups in the extracts.

**DISCUSSION**

**DNA-binding activity**

DNA-binding secondary metabolites are of current importance for the discovery of new drugs. The emerging new diseases today have increased the activity in the exploration of secondary metabolites with DNA-binding activity from natural products. Many anticancer, antibiotic and antiviral drugs exert their primary biological effects by reversibly interacting with nucleic acids. Therefore, these biomolecules represent a major target in drug development (Haq, 2002). The study of Maier et al. (1999a) on the biomolecular-chemical screening of extracts from *Streptomyces* and *fungi imperfecti* revealed new DNA-binding metabolites and validated the so-called “biomolecular-chemical screening” as reported in their study. Mushrooms are promising sources of bioactive secondary metabolites and there is a need to tap these metabolites for potential bio-prospecting. In fact, the results of this study suggest a rich reserve of bioactive molecules in mushroom species.

Studies show that the mechanism of action of anticancer compounds is through DNA-binding. For example, Irofulven, a toxic constituent of the jack-o-lantern mushroom (*Omphalotus olearius*) is rapidly absorbed by tumor cells, and once inside the cells, the compound binds to DNA and protein targets and the binding interferes with DNA replication and cell division of tumor cells, leading to tumor-specific apoptotic cell death (UniSci, 2001). The study of Jeong et al. (2008) demonstrated the antitumor and immunomodulating effects of exo-biopolymer (EXP) produced by *G. applanatum* in mice. EXP significantly inhibited the growth of solid tumor and increased the natural killer (NK) cell activity in a dose-dependent manner (Jeong et al., 2008).

Antibiotic and antifungal compounds are also known as DNA-binders. For example, a new bisintercalating anthracycline antibiotic binds with high affinity to DNA (Chaires, 1998). Another is the antitumor antibiotic
mithramycin A (MTA) which is a DNA minor groove binding ligand. It binds to G/C-rich tracts as a dimer that forms in the presence of divalent cations such as Mg (2+) (Barcelo et al., 2007). Antitumor, antineoplastic, antimalarial, antibiotic and antifungal agents bind to DNA as intercalators or groove binders, and therefore can perturb cellular processes such as transcription (Palchaudhuri et al., 2007). Therefore, the DNA-binding activity G. applanatum (Pers.) Pat. can be explored for the development of anti-cancer drugs or as molecular inhibitors against pathogenic microorganisms.

There are previous reports on the biological activities of secondary metabolites from G. applanatum (Pers.) Pat. such as antimitogenic (Lakshmi, 2006), antitumor and immunomodulating activity (Jeong et al., 2008; Sasaki et al., 1971), cholesterol synthesis inhibitors (Hajjaj, 2005), antioxidant, antimicrobial, antiviral, antiallergic, anti-inflammatory, antiatherogenic, hypoglycemic, hepatoprotective and anti-HIV activities (Sanodiya et al., 2009; Sheena et al., 2005; Lindequist et al., 2005). Results of these studies indicate that the metabolites present in G. applanatum (Pers.) Pat. are DNA-binders since these biological activities are associated with DNA-binding.

Results of the present study are a step further compared to previous studies because of the non-solvent extraction method employed and the partial chemical characterization of the bioactive secondary metabolites from wild G. applanatum (Pers.) Pat. from Bukidnon Province, Philippines. This is significant since DNA-binding is an important characteristic for the discovery of antitumor and anticancer drugs, and certain antibiotics. However, the complete chemical elucidation of the separated metabolites would be necessary to match specific potential targets and pathology in humans, should the extracts be developed as drugs. NMR analysis of purified metabolites would be the next logical step to be taken in this study as part of the researchers' recommendations.

In vivo cytotoxicity

Cytotoxicity study is an important step in developing a certain pharmaceutical product. The cytotoxic activity of natural products may suggest that they are outright toxic or they have potential anticancer activity. Brine shrimp lethality assay has been used as a pre-screen to evaluate the toxicity of natural products. Authors initially found that there is a positive correlation between brine shrimp toxicity and human tumor cell lines cytotoxicity and they are exploiting this easy and inexpensive test to isolate natural products with antitumor or anticancer activity. Bioactive compounds are almost always toxic in high doses and BSLA determines the lethal concentrations of active compounds in brine medium (McLaughlin et al., 1998).

In the present study, all SC-CO₂ extracts of G. applanatum (Pers.) Pat. were shown to be biologically active. However, these extracts exhibited varied cytotoxic activities towards the brine shrimp nauplii. The observed 100% mortality of brine shrimp nauplii at 1000 µg/ml is in agreement with Chairul et al. (1990) who reported that some components of G. applanatum (Pers.) Pat. such as esters are toxic at high concentrations. The FT-IR spectra of the extracts showed the presence of ester at high concentration as revealed by the sharp absorption peaks at 1741 and 1165 cm⁻¹. From this result, other bioactivities of the local strain of G. applanatum (Pers.) Pat. from Bukidnon Province, Philippines are expected since the extracts tested are biologically active.

Results o
Purification and isolation by means of chromatography is also widely used for natural products. For instance, RP-HPLC was used for the recovery and purification of ganoderic acids from triterpene-enriched extracts of *G. lucidum* mycelia (Tang et al., 2006). Furthermore, triterpenoids and other compounds extracted from *G. lucidum* had been separated and characterized using RP-HPLC. HPLC profiles could also be used in determining the chemical characteristics of the extract which can be helpful in chemical identification. In fact, HPLC technique has been used to determine certain compounds on the basis of their retention time in HPLC chromatograms provided a standard compound is run in parallel. In the present study, the three SC-CO$_2$ extracts from *G. applanatum* were subjected to RP-HPLC and the resulting chromatograms were analyzed.

In this study, chromatograms of the extracts showed very slight differences. They almost share the same profile. The highest peak is apparent in the chromatograms. These dominant peaks showed retention time at 7 to 8 min, indicating that they share some common chemical property such as polarity. This is supported by FTIR data. It should be stressed out that reverse-phase HPLC was used for this study. According to Hsu et al. (2001), the characteristic of a reverse phase column is that components which are highly polar can be eluted easier than less or nonpolar components. Therefore, the highly polar columns have the shorter retention time in the chromatogram. It should be noted that in the present study, the chromatograms showed peaks with higher absorbance in short retention times (7 to 8 min). This result suggests that the extracts are highly polar.

The highest peaks represent chemical compounds that could be isolated for identification and structural elucidation. Furthermore, it is noted that the varying degrees of applied pressure used in SC-CO$_2$ extraction efficiently fractionates the active principles of *G. applanatum* (Pers.) Pat. to a high level of purity. Apparently, this is what can be seen in the HPLC patterns of extracts.

**FTIR spectroscopy**

Unlike synthetic drugs, natural products are complicated system of chemical mixtures. To obtain a characteristic fingerprint of natural products that will determine the presence of chemical constituents, spectroscopical methods are helpful. FTIR is proven to be a useful tool for elucidating structures and composition of chemical compounds (Gasparri and Muzio, 2003). Since FTIR spectroscopy can reveal chemical compositions and their structures, this technique has been widely used in traditional Chinese medicines (TCM) investigations recently (Cheng et al., 2010). For instance, different *G. lucidum* products have been studied by FTIR spectroscopy (Sun et al., 2001). In the present study, FTIR spectroscopy analysis of the SC-CO$_2$ extracts of *G. applanatum* (Pers.) Pat from Bukidnon Province, Philippines had been accomplished.

The study of Wang et al. (2012) on *G. lucidum* spores by FTIR microspectroscopy revealed peaks which are similar to the present study. These peaks are those that correspond to the asymmetric and symmetric stretching of CH$_2$ and CH$_3$, C = O stretch, esters, and rocking vibration and bending of CH$_3$. The IR spectra of the crude exopolysaccharide from *G. lucidum* (Mahendran et al., 2012) also showed similarity with the IR spectra of the present study, such as the presence of OH and carbonyl stretching. Choong et al. (2011) also reported functional groups such as CH$_2$ asymmetric stretches and C = O groups present in *G. lucidum*. In addition, bioactive compounds from *G. applanatum* have been isolated, purified and identified (Muhsin et al., 2011). IR spectra showed absorbency bands of O-H, CH, CH$_2$ and C = O, functional groups that are also present in this study. The absorption bands of the IR spectra in this study were compared with the IR spectra of the G1 and G2 compounds isolated from *G. applanatum* from Southern Iraq by Muhsin et al. (2011). These G1 and G2 were identified as member of tannin and terpenoids groups, respectively (Muhsin et al., 2011). The similarities of the IR data from the present and previous studies on *Ganoderma* suggest that common bioactive compounds such as fatty acids, sterols, and proteins can be isolated from this group of basidiomycete. However, structure elucidation of the compounds present in the SC-CO$_2$ extracts of *G. applanatum* used in this study is necessary for complete identification. Furthermore, the present study showed the significance of the use of FTIR for the study of TCM.

**Conclusion**

In conclusion, the *G. applanatum* (Pers.) Pat. from Bukidnon Province, Philippines is a potential source of biologically active DNA-binding compounds. Its narrow cytotoxicity towards the brine shrimp *A. salina* Leach may warrant its safety for human use. However, the components responsible for these biological activities are currently only partially characterized. Therefore, further investigation should be performed on the complete identification of compounds responsible for the reported activities, especially the DNA-binding activity. This can be done through nuclear magnetic resonance (NMR) studies and direct anti-tumor assays.

**ACKNOWLEDGEMENTS**

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