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# Anti-inflammatory and anticancer activities of extracts and compounds from the mushroom *Inonotus obliquus*

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ABSTRACT

Mushroom *Inonotus obliquus (I. obliquus)* has been used as functional food and traditional Chinese herbs for long time. An efficient method for bioassay-guided preparative isolation was used for identifying the anti-inflammatory and anticancer constituents in *I. obliquus*. The petroleum ether and ethyl acetate fractions were found to have significant inhibition effects on NO production and NF-κB luciferase activity in macrophage RAW 264.7 cells and cytotoxicity against human prostatic carcinoma cell PC3 and breast carcinoma cell MDA-MB-231. Six main constituents were isolated from these two fractions and they were identified as lanosterol (1), 3β-hydroxy-8,24-dien-21-al (2), ergosterol (3), inotodiol (4), ergosterol peroxide (5) and trametenolic acid (6). Compound ergosterol, ergosterol peroxide and trametenolic acid showed anti-inflammatory activities and ergosterol peroxide and trametenolic acid showed obviously cytotoxicity on human prostatic carcinoma cell PC3 and breast carcinoma MDA-MB-231 cell. The results obtained in this work might contribute to understanding the biological activity of mushroom *I. obliquus* for food and drug application.



## 1. Introduction

Mushroom *Inonotus obliquus* (*I. obliquus*) was a white-rot fungus belonging to the family of *Hymenochaetaceae Donk*, habiting as a parasitism on birches in the cold latitudes of Europe and Asia (Hawksworth, Kirk, Sutton, & Pegler, 1995). In Russian, *I. obliquus* had been used as a traditional remedy to cure various diseases such as cancer, cerebrovascular diseases, diabetes, gastrointestinal diseases since the sixteenth century (Choi et al., 2010; Sun, Ao, & Lu, 2008). Triterpenes, polysaccharides, polyphenols and melanin were found in *I. obliquus*, which were responsible for the anticancer and antitumor activities (Handa, Yamada, & Tanaka, 2012; Song et al., 2008), anti-inflammatory ability (Van et al., 2009), antioxidant effect (Ma, Chen, Zhang, Zhang, & Fu, 2012), hypoglycemic ability (Lu, Chen, Dong, Fu, & Zhang, 2010), immunomodulatory activity (Fan, Ding, Ai, & Deng, 2012) and anti-mutagenic properties (Ham et al., 2009). However, most of the bioactive studies were mainly focused on the extracts or fractions and the effective constituents were not well illustrated.

Chronic inflammation was implicated in the pathogenesis of series of diseases including atherosclerosis, obesity, metabolic syndrome, diabetes, neurodegenerative diseases, and even several types of cancers (Moro et al., 2012). Macrophagesacted as a crucial role in the inflammatory response and it could release a variety of factors including nitric oxide (NO), prostaglandin mediators and proinflammatory cytokines (TNF-α, IL-1β, IL-6) in response to activating stimulus such as lipopolysaccharide (LPS). Many kinds of natural products were investigated on the anti-inflammatory properties using the LPS-induced macrophage model. *I. obliquus* was a traditional medicinal mushroom and it had been reported to have anti-inflammatory potential on the extracts. Several studies on methanol extract and ethanol extract from *I. obliquus* had shown to inhibit macrophage functions by decreasing the production of inflammatory mediators such as NO, prostaglandins ($PGE_2$) and some cytokines (Kim et al., 2007; Park et al., 2005; Van et al., 2009). However, there were no reports concerning the active compounds on the anti-inflammatory activity. Steroids and triterpenes compounds had been reported to show significantly anti-inflammatory ability in other natural resources (Chang, Wen, Wang, & Duh, 2008; Jiang & Dusting, 2003). However, the anti-inflammatory activities of triterpenes compounds in *I. obliquus* were still unknown.

Anticancer experiments with *n*-hexane extract and water extract of *I. obliquus* had been conducted and the extracts were found to exhibit anticancer effects (Kahlos, Kangas, & Hiltunen, 1987; Youn et al., 2008). As the main chemical constituents of *I. obliquus*, lanostane-type triterpenes compounds were found to have potential anticancer abilities. Lanostane-type triterpenoids isolated from the sclerotium of *I. obliquus* such as inotodiol (De & Ourisson, 1972), 3β,22dihydroxylanosta-7,6(11),24-triene, 3β-hydroxylanosta-8,24-dien-21-al (Kirsti, Lauri, & Raimo, 1987),

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22R-epoxylanost-8ene-3β,24S-diol, trametenolic acid, lanosterol (Nakata et al., 2007), inonotsulides A, B, and C, inonotsuoxides A and B, inonotsutriols A, B, and C, lanosta-8,23E-diene-3β,22R,25-triol and lanosta-7:9(11), 23E-triene-3β,22R,25-triol, spiroinonotsuoxodiol, inonotsudiol A and inonotsuoxodiol A, and inonotsutriols D and E (Handa et al., 2012) were reported to show anti-tumor promoting activities. Inotodiol was found to have a significant anticancer activity on Walker 256, MCF-7 and Hela S3 tumor cells (Kahlos et al., 1987; Rzymowska, 1998). Although some studies on the anti-cancer effects of water, methanol, petroleum ether, *n*-hexane extracts and some lanostane-type triterpenoids from *I. obliquus* against Walker 256, Hela S3, S180 cells were reported, anticancer effects using bioactivity-guided screening method against the human prostatic carcinoma cell PC3 and breast carcinoma cell MDA-MB-231 had not been found in literature.

In this study, the purpose was to isolate and identify the bioactive fraction and compounds from *I. obliquus* by using activity-guided isolation methods. The anti-inflammatory and anti-cancer capacities of ethanol extract (EE), petroleum ether fraction (PEF), ethyl acetate fraction (EAF), *n*-butyl alcohol fraction (*n*-BF), water fraction (WF) and six compounds were comparatively studied and the structure–activity relationship of the six compounds was also discussed.

## 2. Materials and methods

### 2.1. Materials and chemicals

The sclerotia of *I. obliquus* were purchased from the Northeast Natural Products Trading Company (Haerbin, China). Voucher specimens (No. TJC 200702) were deposited at the School of Pharmaceutical Science and Technology, Tianjin University. Murine macrophage cells RAW264.7, human prostatic carcinoma cells PC3 and breast carcinoma MDA-MB-231 cell were obtained from the American Type Culture Collection (Rockville, MD, USA). RPMI 1640, phosphate buffered saline (PBS), lipopolysaccharide (*Escherichia coli*, serotype 0127: B8; LPS), and dimethyl sulfoxide were acquired from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Geneticin (antibiotic G-418) was purchased from Gibco BRL (Grand Island, NY, USA). All of the solutions and buffers were prepared with deionized water.

### 2.2. Extraction and isolation

The sclerotia of *I. obliquus* (2.5 kg) was ground to a fine power using a laboratory mill (60–200 mesh), followed by extraction with ethanol (50 L) for 2 h at 78 °C for three times. The collected ethanol extracts were then combined, concentrated under reduced pressure and the resulting fractions were dried in vacuum. The final yield of ethanol extract (EE) was 405 g. The aliquot (400 g) of the ethanol extract was partitioned into a P.E.–$H_2O$ (1:1, v/v) mixture to furnish a P.E.-soluble fraction (PEF, 6 g) and an aqueous phase. The aqueous phase was further extracted with EtOAc to give an EtOAc-soluble fraction (EAF, 50 g), followed by *n*-BuOH to give a *n*-BuOH fraction (*n*-BF, 98 g) and $H_2O$-soluble fraction (WF, 240 g).

PEF (5 g) was subjected to ordinary-phase silica gel column chromatography [500 g, P.E.–EtOAc (100:0 → 90:10 → 80:20 → 70:30 → 50:50)] to give 14 fractions (Fr.1 ∼ Fr.14). Fraction 4 was recrystallized using MeOH to give compound **1** (lanosterol). Fraction 6 was further subjected to ordinary-phase silica gel column chromatography [P.E.–EtOAc (90:10 → 80:20 → 70:30 → 50:50)] to give 6 fractions [Fr. 6-1, Fr. 6-2, Fr. 6-3, Fr. 6-4 (compound **2**, 3β-hydroxy-8,24-dien-21-al), Fr. 6-5, and Fr. 6-6]. Fraction 7 was isolated using ordinary-phase silica gel column chromatography [P.E.–EtOAc (90:10 → 80:20 → 70:30 → 50:50)] to give 5 fractions [Fr. 7-1 (compound **3**, ergosterol), Fr. 7-2, Fr. 7-3, Fr. 7-4 (compound **4**, inotodiol), and Fr. 7-5]. Fraction 9 was isolated with ordinary-phase silica gel column chromatography [P.E.–EtOAc (85:15 → 70:30 → 50:50)] to give compound **5** (ergosterol peroxide).

EAF (45 g) was subjected to ordinary-phase silica gel column chromatography [1000 g, $CH_2Cl_2$: MeOH (100:0 → 98:2 → 90:10 → 80:20 → 70:30 → 50:50 → 0:100)] to afford 20 fractions (Fr.1 ∼ Fr.20). Fraction 4 was subjected to ordinary-phase silica gel chromatography [P.E.–EtOAc (90:10 → 80:20 → 70:30 → 50:50)] to give 7 fractions [Fr. 4-1 (compound **1**, lanosterol), Fr. 4-2, Fr. 4-3, Fr. 4-4, Fr. 4-5, Fr. 4-6, and Fr. 4-7]. Fraction 7 was isolated with ordinary-phase silica gel column chromatography [P.E.–EtOAc (9:1 → 5:1 → 3:1 → 1:1)] to furnish 4 fractions [Fr. 7-1, Fr. 7-2, Fr. 7-3 (compound **2**, 3β-hydroxy-8,24-dien-21-al), and Fr. 7-4]. Fraction 14 was further subjected to ordinary-phase silica gel chromatography [P.E.–EtOAc (4:1 → 2:1 → 1:1 → 1:2)] to afford 16 fractions (Fr. 14-1 ∼ Fr. 14-16). Fraction 14-8 was further purified with ordinary-phase silica gel chromatography [P.E.–EtOAc (4:1 → 2:1 → 1:1 → 1:2)] to give 3 fractions (Fr. 14-8-1, Fr. 14-8-2, and Fr. 14-8-3), followed by recrystallizing fraction 14-8-1 to obtain compound **6** (trametenolic acid). Fraction 14-8-3 was further purified using ordinary-phase silica gel chromatography [P.E.–EtOAc (5:1 → 3:1 → 1:1 → 1:2)] to give compound **4** (inotodiol). Compounds **1**–**6** were identified by comparison of their spectral data ($^1$H NMR, $^{13}$C NMR, and MS) with reported values, and had the purity over 98%.

### 2.3. Anti-inflammatory assays

#### 2.3.1. Cell culture

Murine macrophage RAW 264.7 cell lines were maintained and cultured at 37 °C under humidified air, with 5% CO2 atmosphere in RPMI1640 (GIBCO Invitrogen Corporation, Grand Island, NY, USA) supplemented with 10% foetal bovine serum (FBS), 100 units/mL penicillin, 100 mg/mL streptomycin and 1.176 g/L sodium bicarbonate.

#### 2.3.2. Nitrite assay

Exponentially growing RAW 264.7 macrophages were plated in 96-well plates at a density of $1 \times 10^5$ cells per well in 400 μL of culture medium, allowing them to adhere overnight. Cells were then treated or not with positive control celastrol, or samples dissolved in DMSO, and incubated at 37 °C, 5% $CO_2$ for 24 h. The final concentration of solvent in the culture medium was maintained at 0.5% (v/v) in order to avoid solvent toxicity. Cells were then stimulated with LPS (100 ng/mL). Cell-free supernatants were collected, stored at −80 °C and underutilised to measure NO content using the Griess reaction (Green, Meltzer, Hibbs, & Nacy, 1990) with minor modifications after 24 h. In brief, 100 μL aliquots of cell supernatants were incubated with 50 μL of 1% sulfanilamide and 50 μL of 0.1% *N*-1-naphtylethylenediamine dihydrochloride in 2.5% $H_3PO_4$ at room temperature for 20 min. Absorbance at 570 nm was then measured using an automated 96-well Varioskan Ascent plate reader (Thermo Electron) and nitrite levels in the samples were calculated from a standard curve with known concentrations of sodium nitrite.

#### 2.3.3. NF-κB luciferase assay

It is accepted that luciferase can be used as measurement of the activation (high light incidence) or inhibition (low light incidence) of NF-κB. Details of the experiments can be found in the study of Bremner et al. (2009). Briefly, RAW264.7 cells stably transfected with the NF-κB reporter gene were plated in 96-well plates. Following a 24 h recovery period, the cells were treated with extract, fractions and compounds for an additional 18 h in the presence of

LPS (100 ng/mL). To determine NF-κB luciferase activity, the Luciferase Reporter Assay System purchased from Promega (Madison, WI) was used. Cell lysates (15 μL) from treated RAW264.7 cells were placed in opaque 96 well plates. Luciferase Assay Reagent (50 μL) was injected and samples were read by a fluorometer (LMAX 2, Molecular devices). Negative (resting cells without stimulation) controls were included to monitor assay consistency. A positive control of celastrol was also employed to ensure the assay responded to a known inhibitor of NF-κB.

### 2.4. Measurement of anticancer activities in vitro cytotoxicity against human cancer cell lines

The human prostatic carcinoma cells PC3 and breast carcinoma cell MDA-MB-231 were used for the anticancer assays. These cell lines were maintained with RPMI1640 (GIBCO Invitrogen Corporation, Grand Island, NY, USA) as described above for the RAW264.7 cells. Cytotoxicity against various human cancer cells was assessed using the resazurin reduction test as described by Dufour et al. (2007) on 96-well tissue culture plates. Exponentially growing cells including PC3 and MDA-MB-231 were plated at a density of $5 \times 10^3$ cells per well in 96-well plates in 100 μL of culture medium and were allowed to adhere for 16 h before treatment. Then100 μL of extract, fractions or pure compounds dissolved in the appropriate solvent (DMSO) were added. In order to avoid solvent toxicity, the final concentration of solvent in the culture medium was maintained at 0.5% (v/v). The cells were allowed to grow for 72 h in the absence or in the presence of samples (37 °C, 5% CO2, 90% RH). The absorbance at 590 nm was measured using an Emax microplate reader (Molecular Devices, Sunnyvale, CA, USA). Cytotoxicity was expressed as the concentration of extracts or compounds inhibiting cell growth by 50% ($IC_{50}$).

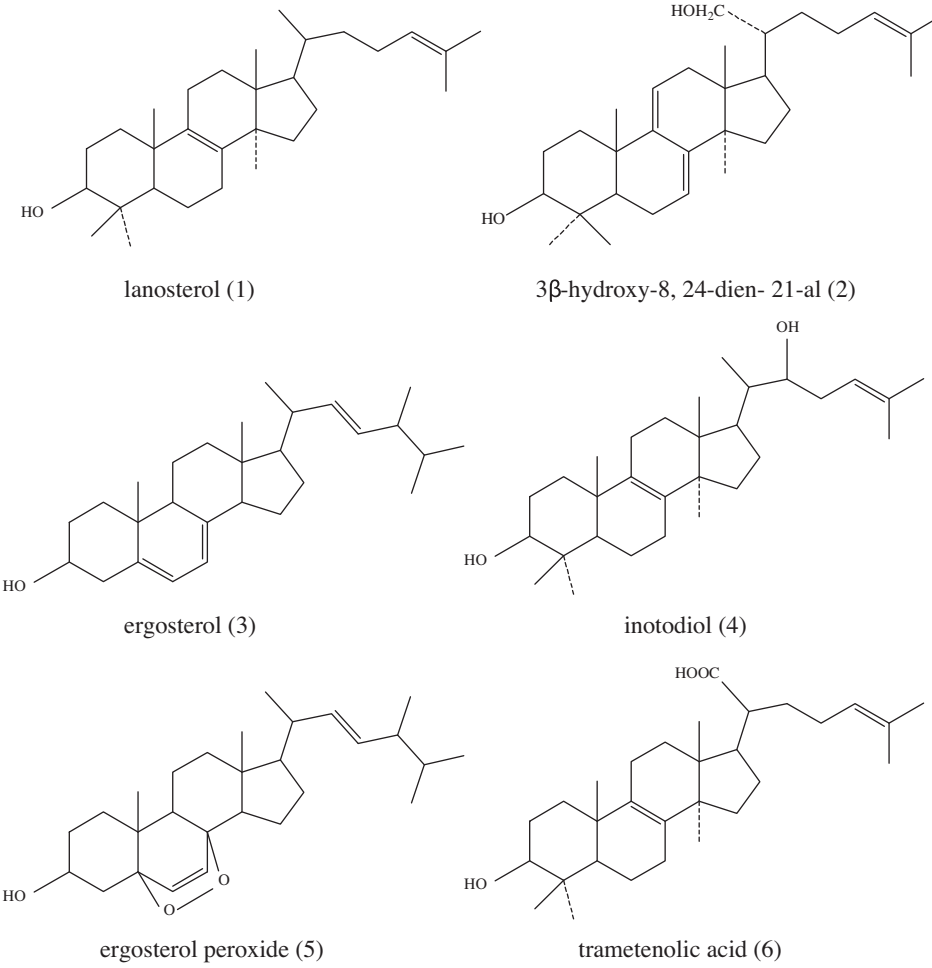
### 2.5. Statistical analysis

The results are presented as means ± standard error of the mean (SEM). Differences in mean values between groups were analysed by a one-way analysis of variance (ANOVA) and Student's *t*-test using SPSS statistical software (version 16.0 for Windows, SPSS Inc., Chicago, IL, USA), the statistical significance of mean differences was based on a $p$ value of <0.05.

## 3. Results and discussion

### 3.1. Extraction and isolation

Sclerotia of *I. obliquus* was extracted and fractioned to afford EE (16.2%), PEF (0.24%), EAF (2%), *n*-BF (3.92%) and WF (9.6%), and the fractions PEF and EAF were then separated with a series of silica-gel column chromatography. Six known triterpenes were obtained and identified as lanosterol, 3β-hydroxy-8,24-dien-21-al, ergosterol, inotodiol, ergosterol peroxide and trametenolic acid based




lanosterol (1) 3β-hydroxy-8, 24-dien- 21-al (2)

ergosterol (3) inotodiol (4)

ergosterol peroxide (5) trametenolic acid (6)

**Fig. 1.** Structure of six compounds (**1**–**6**).

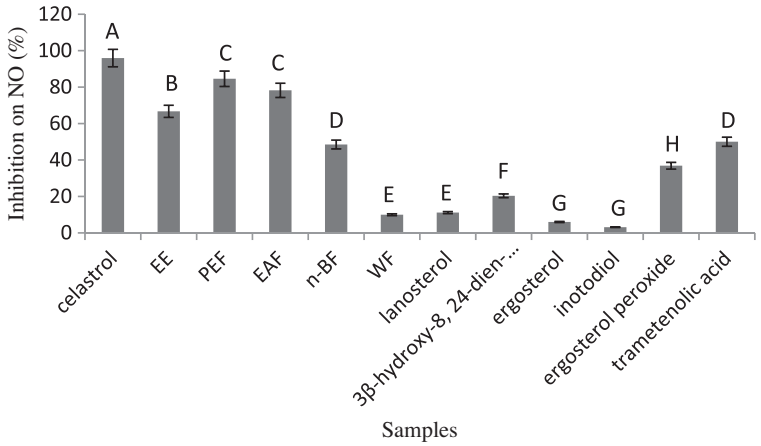



**Fig. 2.** Inhibitory effect on NO production of extract, fractions and compounds from *I. obliquus* in LPS-induced RAW 264.7 macrophages. Values sharing the same letter are not significantly different at $p < 0.05$.

on their physical and spectral data that showed good agreement with published references (Lu, Chen, Dong, Fu, & Zhang, 2010; Mallavadhani et al., 2006). The structures of six compounds were presented in Fig. 1.

### 3.2. Effect of the extract, fractions and compounds on NO production in RAW 264.7 macrophages

Stimulation of RAW 264.7 macrophages by LPS induced iNOS and overproduction of NO, which could be detected and quantified photometrically. Results presented in Fig. 2 showed that positive control celastrol (25 μg/mL) significantly inhibited NO release by the rate of 95.94%. All extract and fractions significantly inhibited NO release and decreased in the order of PEF (84.59%), EAF (78.24%), EE (66.72%) and *n*-BF (48.52%) except of WF (9.99%) at the concentration of 40 μg/mL. It was noted that fraction PEF exhibited the most active anti-inflammatory effect among all extract and fractions. Park et al. (2005) reported that methanol extracts from *I. obliquus* showed an inhibitory effect on LPS-induced NO production in a dose-dependent manner, with an $IC_{50}$ value of 89 μg/mL. In this study, PEF and EAF showed better anti-inflammatory ability than that of EE and methanol extracts. Inhibitory rates of compounds **1**–**6** on NO production decreased in a turn of trametenolic acid (50.04%), ergosterol peroxide (36.88%), 3β-hydroxy-8,24-dien-21-al (20.36%), ergosterol (6.00%) and inotodiol (3.13%).

### 3.3. Inhibition of extract, fractions and compounds on NF-κB luciferase activity

The results of NF-κB luciferase activity analysis was depicted in Fig. 3. LPS treatment had activated and enhanced the activity of luciferase as compared to control. NF-κB regulated many signaling pathway in inflammation and cancer. Series of genes involved in inflammation and tumorigenesis were influenced by NF-κB and they were easily activated by inflammatory stimuli like LPS (Shishodia & Aggarwal, 2004). Results showed that celastrol (250 μg/mL) significantly inhibited NF-κB luciferase activation by 93.83%. It was noted that PEF (95.94%) and EAF (96.64%) could reduce the activity of NF-κB luciferase, which were more powerful than that of celastrol. There were no obvious inhibitory effects found in the ethanol extract and fractions *n*-BF and WF. Compounds ergosterol peroxide (53.63%), ergosterol (23.46%) and trametenolic acid (18.42%) showed a relative effective NF-κB luciferase inhibitory potential, while lanosterol, 3β-hydroxy-8,24-dien-21-al and inotodiol did not exhibit any NF-κB inhibition ability. The results showed that PEF and EAF were the most potential NF-κB luciferase inhibitor. Ergosterol peroxide from PEF was testified as the most active NF-κB luciferase inhibition compound, indi-

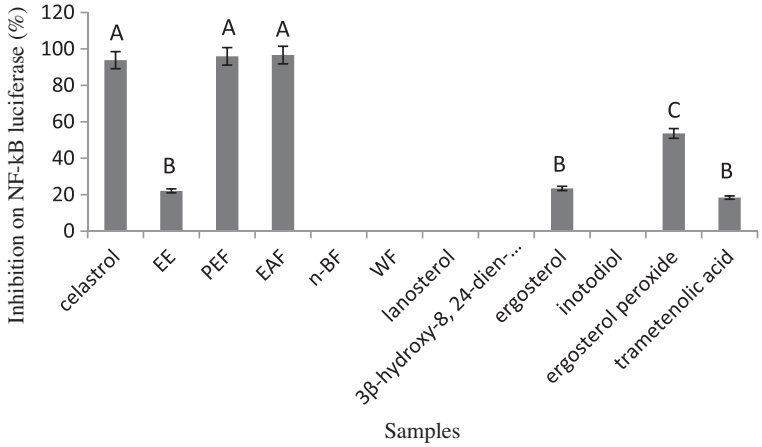



**Fig. 3.** Inhibitory effect on NF-κB luciferase of extract, fractions and compounds from *I. obliquus* in LPS-induced RAW 264.7 macrophages. Values sharing the same letter are not significantly different at $p < 0.05$.

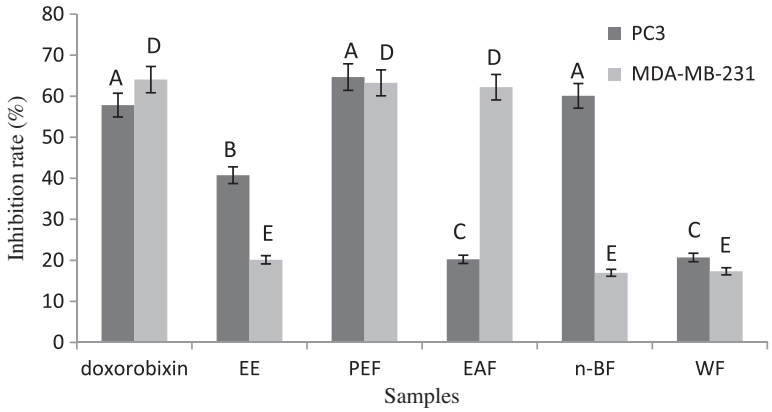



**Fig. 4.** Cytotoxic activities of extract and fractions from *I. obliquus*. Values sharing the same letter are not significantly different at $p < 0.05$.

cating that the anti-inflammatory properties of PEF and EAF might be due to the presence of triterpenes. In Van's study (2009), ethanol fraction, water-soluble polysaccharides fraction and polyphenol fraction from *I. obliquus* were found to have inhibitory effects on pro-inflammatory cytokine release. It was showed that ethanol fraction did not reduced IL-1β levels but significantly reduced IL-6 and TNFα levels. However, there was no report on the inhibition on NF-κB luciferase activities in literature for *I. oblquus*.

### 3.4. Evaluation of cytotoxicity against tumor cell lines

Cancer had been a formidable problem for people for centuries. Many chemical compounds from natural resources had been investigated and identified as inhibitors to various cancer cells. *I. obliquus* had long been used to evaluate its anticancer properties from 16th century (Kirsti, Lauri, & Raimo, 1986). In this study, the anticancer activity of EE, PEF, EAF, *n*-BF, and WF was evaluated against prostatic carcinoma cell line PC3 and breast carcinoma cell line MDA-MB-231. The inhibitory rates of the extract and fractions on various tumor cells were shown in Fig. 4. Among all extract and fractions, PEF was the most active against PC3 and MDA-MB-231 with inhibitory rates of 64.66% and 63.26%, while the inhibitory rates of positive control doxorobixin were 57.84% and 64.05%, respectively. EAF showed a relatively active cytotoxic activity against PC3 and MDA-MB-231 with the inhibition rates of 20.25% and 62.20%, respectively. Therefore, the fractions PEF and EAF were chosen for further isolation and evaluation of their anticancer activities.

Six main compounds were isolated from fractions PEF and EAF and the cytotoxicity against prostatic carcinoma cell line PC3 and breast carcinoma cell line MDA-MB-231 cell line were assayed. The IC50 values obtained were shown in Table 1. PEF and EAF were found to be active against PC3 and MDA-MB-231 with IC50 values of 29.57 ± 12.18 μg/mL and 19.22 ± 0.46 μg/mL, 57.39 ± 14.46 μg/mL and 46.49 ± 13.21 μg/mL, respectively. These results suggested that fractions from *I. obliquus* had an active influence on proliferation of both cell lines and targeted carcinoma cell line PC3. Among the six isolated compounds assayed, the IC50 values against PC3 were calculated to be 9.82 ± 0.98 μM for ergosterol, 38.19 ± 1.67 μM for ergosterol peroxide, 63.71 ± 3.31 μM for trametenolic acid, and 73.46 ± 0.64 μM for 3β-hydroxy-8,24-dien-21-al, while inotodiol and lanosterol were found to be inactive against PC3 with the IC50 value higher than 100 μM. 3β-hydroxy-8,24-dien-21-al, ergosterol peroxide and trametenolic acid showed active cytotoxic activities against MDA-MB-231 with IC50 values of 36.50 ± 1.13 μM, 30.23 ± 3.24 μM and 55.03 ± 5.40 μM, respectively, indicating that ergosterol peroxide was the most active constituent among the six compounds detected. Ergosterol, inotodiol and lanosterol were found to be inactive in inhibition growth of MDA-MB-231 cell with the IC50 values higher than 100 μM. The active cytotoxic activities against PC3 and MDA-MB-231 of PEF and EAF could be due to the presence of triterpene compounds. These results suggested that PEF and EAF from *I. obliquus* could be considered as potential sources of anticancer compounds.

**Table 1**
Cytotoxic activities against PC3 and MDA-MB-231 of extract, fractions and compounds from *I. obliquus*.

| Sample | $IC_{50}$ (μM)[a] | |
|---|---|---|
| | PC3 | MDA-MB-231 |
| PEF (μg/mL) | $29.57 \pm 12.18^{B}$ | $57.39 \pm 14.46^{B}$ |
| EAF (μg/mL) | $19.22 \pm 0.46^{B}$ | $46.49 \pm 13.21^{AB}$ |
| Lanosterol | >100 | >100 |
| 3β-Hydroxy-8,24-dien-21-al | $73.46 \pm 0.64^{C}$ | $36.50 \pm 1.13^{A}$ |
| Ergosterol | $9.82 \pm 0.98^{A}$ | >100 |
| Inotodiol | >100 | >100 |
| Ergosterol peroxide | $38.19 \pm 1.67^{B}$ | $30.23 \pm 3.24^{A}$ |
| Trametenolic acid | $63.71 \pm 3.31^{C}$ | $55.03 \pm 5.40^{B}$ |

$IC_{50}$ value was defined as the concentration of extracts and compounds that caused 50% inhibition of PC3 and MDA-MB-231 cells growth in vitro.
Values in the same column sharing the same letter are not significantly different at $p < 0.05$.
[a] Data are expressed as mean ± SEM of three independent experiments.

There were some studies on compounds from *I. obliquus* on other cancer cell lines. Kirsti et al. (1987) reported that inotodiol possessed active cytotoxic activity against MCF-7 and could inhibit proliferation at the rate of 100%. Nakata et al. (2007) isolated inonotsuoxides A, inotodiol, trametenolic acid and lanosterol from *I. obliquus* and found that inotodiol exhibited the potent anti-tumor promoting activity in the vivo carcinogenesis test. However, inotodiol did not show active cytotoxic ability against PC3 and MDA-MB-231 cells in the present study. So the cytotoxicity of the compound inotodiol might be selective.

It was accepted that cancer was always associated with inflammatory responses and the expression of inflammatory genes was often negatively correlated with cancer stage and prognosis (Kim, Moon, Kim, Choi, & Lee, 2012). Inflammation played a crucial role in cancer progress. Extract, fractions and compounds from *I. obliquus* were evaluated on their anti-inflammatory and anticancer effects with the purpose of better understanding their relationship and the most effective constituents. In this study, PEF and EAF significantly reduced the production of LPS-induced pro-inflammatory factor NO and NF-κB in RAW 264.7 cells. Compounds ergosterol peroxide and trametenolic acid showed a relatively good anti-inflammatory properties. It was reported that the production of NO in tissues could contribute to the carcinogenesis process

(Liu & Hotchkiss, 1995; Tamir & Tannenbaum, 1996) because overproduction of NO could lead to enhanced replication of genes and oxidative damage to DNA. In the present study, PEF and EAF not only significantly inhibited NO production and the activity of NF-κB luciferase, but also effectively exerted cytotoxicity against carcinoma cell line PC3 and breast carcinoma cell line MDA-MB-231. The main compound ergosterol peroxide and trametenolic acid showed significant inhibitory effects of NO production and inhibited the activity of NF-κB luciferase. The two compounds also exhibited cytotoxicity against the cancer cell lines. The results indicated that there might be some relationship between inhibitory activities on NO production, NF-κB luciferase activity and cytotoxic effects against cancer cell lines, which was not in agreement with the conclusion of Kim et al. (2012). Further studies will be needed to investigate the precise mechanisms of extract, fractions and isolated triterpenes from *I. obliquus* on cytotoxic activities against cancer cell lines and anti-inflammatory activities.

## 4. Conclusion

An efficient method for bioassay-guided preparative isolation was used for identifying the anti-inflammatory and anticancer constituents in *I. obliquus*. Six compounds were isolated and identified. Anti-inflammatory and anticancer effects of extract, fractions and the six isolated compounds **1**–**6** were evaluated. PEF and EAF were found to exhibit effectively anticancer and anti-inflammatory activities, compounds ergosterol peroxide and trametenolic acid were the main bioactive compounds. The results obtained in this work might contribute to understanding the biological activity of mushroom *I. obliquus* for food and drug application.

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