Potential of Terpenoid Bioactive Compound Isolated from Papua Ant Nest as an Alternative Ovarian Cancer Treatment

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Abstract

Objectives: The purpose of this study was to determine anticancer activity of terpenoid bioactive compound isolated from Papua ant nest on ovarian cancer cells in vitro. Methods: This was a laboratory experimental study which aims to determine the potential of the terpenoid bioactive compound isolated from Papua ant nest to inhibit the growth and induce apoptotic process on ovarian cancer cells (SKO-3) in vitro. Result: Terpenoid had capability to inhibit the growth of ovarian cancer cell line (SKO-3) in vitro, with IC50 of 481 ug/ml at 48 hours and 463 ug/ml at 48 hours, respectively. At a concentration of 600 ug/ml, terpenoid was able to induce apoptotic process on ovarian cancer cell lines in vitro with the apoptotic index of 30% at 24 hours, 35% at 48 hours and 37% at 72 hours, respectively. Conclusion: Terpenoid bioactive compound isolated from Papua ant nest had the ability to inhibit the growth and was able to induce apoptotic process on ovarian cancer cell lines (SKO-3) in vitro.

Keywords

Terpenoid, Ovarian Cell Lines, Apoptotic Index

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1. Introduction

Ovarian cancer, one of the most common gynecological malignancies, has an aggressive phenotype and a relatively poor prognosis, peritoneal dissemination and/or retroperitoneal lymph node metastases are found in two-thirds of patients at the time of diagnosis. The highest incidence areas of ovarian cancer are Europe and North America [1]-[4].

The ability of ovarian cancer cells to metastasise to different sites in the body is the major cause of morbidity and mortality among ovarian cancer patients. In the regulation of tumour mass, a balance between rates of cell proliferation and cell death or apoptosis is crucial [5]-[8].

Apoptosis or programmed cell death is a process whereby cells die in a controlled manner in response to specific stimuli and plays an important role in both carcinogenesis and cancer treatment. Many new treatment strategies targeting apoptosis are feasible and may be used in the treatment of ovarian cancer, including natural products as anticancer drugs in several clinical trials. Naturally occurring plant components from traditional herbs a significant source of potential therapeutic compounds for cancer treatment [9]-[12].

The use of natural products as alternative anticancer therapies tends to increase, especially in developing countries. Thus, the study of medicinal plants to treat cancer has also increased to explore their therapeutic effect for cancer treatment [13] [14].

Indonesia is abundant of herbal medicines that can be used for an alternative therapy, such as Papua ant nest. The local people of Papua boiled the ant nest to treat several diseases and it has been used for the treatment of ascaris, cold, hemorrhoids, burn, including cancer. There is still limited scientific evidence to proof the efficacies of Papua ant nest to cure cancer, especially in ovarian cancer [14] [15].

Papua ant nest plant is a herbaceous plant that is new and has potential as an alternative therapy in treating cancer, especially the Myrmecodia species. [13] The Papua ant nest is epiphytic plant of the Rubiaceae family and has 5 genus, but only 2 genus have association with ants. They are Myrmecodia (45 species) and Hypnophytum (26 species), but only Hypnophytum fornicarum, Myrmecodia tuberosa and Myrmecodia pendens have medicinal value (Figure 1) [16].

Soekmanto et al. (2010) have evaluated the anticancer activity of extract from Myrmecodia pendens using some cancer cells derived from both human cervix (HeLa) and canine mammary tumor (MCM) cell lines. It was found that the IC50 value of water extract A was 27.61 ppm (HeLa) and 54.57 ppm (MCM-B2), while water extract B was 29.36 ppm (HeLa) and 74.20 ppm (MCM-B2). These result suggested that the extract of Papua ant nest (Myrmecodia pendens) have the capability to inhibit the growth of HeLa and MCM-B2 cells [13] [16]. On the background of these findings, we attempted to isolate Myrmecodia pendens of Papua ant nest. In the present study, we could isolate the terpenoid bioactive compound from Papua ant nest and found that the terpenoid active compound isolated from Papua ant nest have capability as a strong antioxidant activity [17].

![Figure 1. Papua ant nest.](image-url)
Previously, Darsono ADH et al. (2013) has proved that the terpenoid bioactive compound isolated from Papua ant nest have a strong antimicrobial activity [17]. Therefore, it is very necessary to search for a new potential anticancer agents from this terpenoid bioactive compounds for an alternative ovarian cancer treatment. This research has been carried out to investigate the potential anticancer of terpenoid bioactive compounds on ovarian cancer cell lines (SKOV-3) in vitro.

2. Methods

This study has been carried out from June 2014 to January 2015. The Papua ant nest was collected from Papua, Indonesia and isolated at the Department of Chemistry, Faculty of Mathematic and Natural Science, Padjadjaran University, Bandung, Indonesia.

*Myrmecodia pendens* of Papua ant nest was cleaned, cut, dried, ground and then was extracted with methanol and water. The extraction process was taken over 10 days period (until resulting terpenoids) utilizing liquid ethyl acetate, without water and under 35°C temperature. The following describes the work flow:

The corn of the ant nest (about 3 kg) was extracted by sokletation method using 60 ml ethyl acetate as solvent which then produced ethyl acetate extract. This extract was thickened by a rotatory evaporator to remove the solvent, resulting 30 gr of thick ethyl acetate extract. This extract was further separated using silica gel 60 chromatography with gradient n-heksana as a solvent: ethyl acetate 2.5% (100:0 to 80:20, v/v) produced 9 fractions. The 7th fraction (3.8 gr) was purified by using n-heksana to produce 1.8 gr white crystal. In order to determine the purity of this crystal, an analysis was conducted through thin layer chromatography (TLC) gel 60 F254 with n-heksana: aseton (8:2) as its solvent. The TLC plate was examined under ultraviolet light at 254 and 365 nm. Furthermore, 10% of acid sulphate was applied to the TLC plate in heated ethanol on a hot plate so that the spots can be visualized. Purplish black spots appeared on the heated TLC plate which indicate the terpenoid compounds. The extracts was subjected to column chromatography, then column were eluted with methanol to obtain a terpenoid fraction, followed by isolation process, we obtained the terpenoid bioactive compound. [18]

The Ovarian Cancer Cell Lines (SKOV-3) were obtained from Laboratory of Rajawali Hospital, Bandung, Indonesia. All ovarian cancer cell lines (SKOV-3) were grown in RPMI 1640 supplemented with 10% fetal bovine serum (heat-inactivated at 56°C for 45 minute) and penicillin/streptomicin, in a humidified, 5% CO₂ atmosphere and 37°C incubator. After all the cells were confluent, the cells were counted using a Neubauer Haemocytometer and resuspended in medium at the final concentration of 5 × 10⁵ cells/ml and mixed with DMSO medium and treated with terpenoid at doses 0, 1.562, 3.125, 6.25, 12.5, 25, 50, 100, 200 µg/ml, respectively. Then the cells (SKOV-3) were incubated for 48 hours and 72 hours, respectively. The number of viable cells was ascertained with MTT reaction and measured at ƛ550 nm followed by IC₅₀ calculation [19].

To determine apoptotic process on ovarian cancer cell lines (SKOV-3) after treatment with terpenoid, we used TUNEL assay to calculated the presence of apoptosis, 1 × 10⁶ cell/ml, were treated with the terpenoid at the concentration of 200 µg/ml, 400 µg/ml, 600 µg/ml for 24 h, 48 h and 72 h, respectively at 37°C on plate with cover slips. Following incubation, cover slips were fixed on objective glass and morphology of the cell was analyzed under microscope [19].

3. Results

The result of this study showed that the terpenoid bioactive compound isolated from Papua ant nest had capability to inhibit the growth of ovarian cancer cell lines (SKOV-3) with IC₅₀ of 481 µgr/ml for 48 hours and 463 µgr/ml for 72 hours, respectively. The inhibition activity of terpenoid bioactive compound to the growth of ovarian cancer cell lines (SKOV-3) was shown in both Table 1 and Table 2.

According to this study, terpenoid bioactive compound was able to induce apoptotic process on ovarian cancer cell (SKOV-3). At the concentration of 200 µg/ml, terpenoid was able to induce apoptotic process on ovarian cancer cell lines in vitro with the apoptotic index of 18% at 24 hours, 22% at 48 hours and 2% at 72 hours, respectively. At the higher concentration (400 µg/ml), terpenoid was able to induce apoptotic process on ovarian cancer cell lines in vitro with the apoptotic index of 22% at 24 hours, 24% at 48 hours and 27% at 72 hours, respectively.

Further analysis, at the highest concentration (600 µg/ml), terpenoid was able to induce apoptotic process on ovarian cancer cell lines in vitro with the value of apoptotic index were approximately 30% at 24 hours, 35% at 48 hours and 37% at 72 hours, respectively.
Table 1. Inhibition activity of terpenoid to the growth of ovarian cancer cell lines (SKOV-3) on 48 hours.

| (µg/ml) | Absorbance | Mean | Percentage of living cells | IC₅₀ |
|---------|------------|------|-----------------------------|------|
|         | 1          | 2    | 3          |          |      |
| Starving| 2.4282     | 2.9543| 1.5362     | 2.3062  | 89.19755766 |
| DMSO    | 2.7376     | 2.7113| 2.9216     | 2.7902  | 108.7362908 |
| 0       | 2.6444     | 2.4245| 2.7358     | 2.6016  | 99.99855312 |
| 1.5625  | 2.8950     | 2.9400| 3.0493     | 2.9614  | 114.2648378 |
| 3.125   | 2.8807     | 3.1071| 2.8584     | 2.9487  | 114.6511561 |
| 6.25    | 2.9677     | 2.9603| 2.6332     | 2.8537  | 111.6126979 |
| 12.5    | 2.3695     |      | 3.1169     | 2.7432  | 107.574442 |
| 25      | 2.9861     | 2.8232| 2.5905     | 2.7999  | 107.1852302 |
| 50      |            | 2.8949| 2.5476     | 2.7213  | 105.8924386 |
| 100     |            | 2.5678| 2.7514     | 2.6596  | 102.5826895 |
| 200     |            | 1.3388| 2.8345     | 2.7179  | 85.65268976 |

Table 2. Inhibition activity of terpenoid to the growth of ovarian cancer cell lines (SKOV-3) on 72 hours.

| (µg/ml) | Absorbance | Mean | Percentage of living cells | IC₅₀ |
|---------|------------|------|-----------------------------|------|
|         | 1          | 2    | 3          |          |      |
| Starving| 0.7306     | 2.1380| 1.2461     | 1.3716  | 33.91795 |
| DMSO    | 3.2197     | 3.4863| 3.2903     | 3.3321  | 96.55324 |
| 0       | 3.3819     | 3.7075| 3.1042     | 3.3979  | 99.99887 |
| 1.5625  | -          | 2.7004| 2.4453     | 2.5729  | 71.60826 |
| 3.125   | -          | 3.0182| 3.4857     | 3.2520  | 96.43305 |
| 6.25    | 3.1909     | -    | 2.8291     | 3.0100  | 89.16878 |
| 12.5    | -          | -    | 3.1865     | 3.1865  | 95.32419 |
| 25      | -          | 3.4206| 3.2799     | 3.3503  | 100.6179 |
| 50      | 3.1605     | 3.1336| 3.1091     | 3.1344  | 92.24649 |
| 100     | 2.9646     | 3.8435| 3.2959     | 3.3680  | 101.1207 |
| 200     |            | 2.1169| 2.7225     | 2.4197  | 65.10581 |

The presence of apoptosis (apoptosis index) on ovarian cell lines (SKOV-3) after treatment with terpenoid active compounds isolated from Papua ant nest was shown in Table 3.

4. Discussions

Ovarian cancer is still the most common cause of gynaecological cancer-related mortality. Patients with this disease generally undergo surgery followed by chemotherapy. However, chemotherapy remains the treatment of choice in ovarian cancer, but the emergence of resistance to anticancer drugs, in particular multidrug resistance (MDR), has made many of available anticancer drugs ineffective. Since MDR is a major obstacle in clinical management of ovarian cancer, it is important to design alternative therapy strategies than can be utilized for the treatment of drug-resistant ovarian cancer cells [20] [21].

In the course of our search for new bioactive compound as an alternative ovarian cancer treatment from
Table 3. Apoptotic indexs of ovarian cancer cell lines (SKOV-3) after treatment with terpenoid.

| Group of treatment | Time of observation | 24 hours | 48 hours | 72 hours |
|-------------------|---------------------|----------|----------|----------|
| Positive control   |                     | 35%      | 58%      | 60%      |
| Negative control   |                     | 5%       | 8%       | 9%       |
| 200 µg/ml          |                     | 18%      | 22%      | 22%      |
| 400 µg/ml          |                     | 22%      | 24%      | 27%      |
| 600 µg/ml          |                     | 30%      | 35%      | 37%      |

Indonesian medicinal plants, we isolated and investigated the terpenoid bioactive compound from Papua ant nest. Among the bioactive compound isolated from ant nest, flavoids is the most widely studied, especially in recent years. Meanwhile, the study of terpenoid bioactive compound isolated from Papua ant nest as anticancer for ovarian cell lines has never been done [22] [23].

Based on the citotoxic analysis of terpenoid in this study, we found that terpenoid had antiproliferative activity against ovarian cancer cell line (SKO-3) in vitro. It was also found that terpenoid had capability to induce apoptotic process on ovarian cancer cell lines. These findings indicated the potential of terpenoid bioactive compound isolated from Papua ant nest as anticancer was able to induce apoptotic process on ovarian cancer cell lines (SKO-3) in vitro.

However, the precise antiproliferative activity and apoptosis induction mechanisms of this terpenoid remain unclear, but we believe that the terpenoid induces apoptosis of ovarian cancer cell lines through an intrinsic apoptotic pathway. Moreover, it is reported that the intrinsic pathway requires disruption of the mitochondrial membrane and the release of mitochondrial protein, such as cytochrome-c. Once cytochrome-c is in the cytosol, together with Apaf-1 activates caspase-9, then activates caspase-3, and the latter then induces apoptosis [24]-[26].

Our study demonstrates a possible therapeutic mechanisms of terpenoid which possesses antineoplastic properties in ovarian cancer cell lines with suppression of proliferation and induction of apoptosis. To the best of our knowledge, this is the first report describing the antiproliferative effect and apoptosis induction by terpenoid in ovarian cancer cell lines. Induction of apoptosis has been recognized as one ideal strategy for cancer chemotherapy. Agents with the ability to induce apoptosis in cancer cell have the potential to be used for anticancer therapy and may candidate as alternative ovarian cancer therapy.

5. Conclusion
In summary, there has been an increasing demand for finding newer and safer chemotherapeutic agents. Natural products, in particular plants, have a wide application in cancer chemotherapy. The terpenoid bioactive compound from Papua ant nest is an important traditional medicine with anticancer effects. Regarding the anticancer mechanisms of this terpenoid, its exact target is still unknown and the pharmacological activities remain unclear.

We believe that based on our results, this terpenoid-warrent further studies as anticancer agents in treatment of ovarian cancer and may enable the development of new anti-cancer drugs.

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