Antiproliferative and Antiplasmodial Investigation of *Alphitonia excelsa* and *Arcangelesia flava*

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*Alphitonia excelsa* (Rhamnaceae) and *Arcangelesia flava* (Menispermaceae) are utilized by locals in Samar island for medicinal purposes. Extracts from twigs of *A. excelsa* exhibited antiproliferative activity against the A2780 human ovarian cancer cell line, while a stem extract of *A. flava* had potent activity in an antimalarial screen against the drug-resistant *Plasmodium falciparum* Dd2 strain. Extracts of *A. excelsa* and *A. flava* were subjected to activity-guided isolation using column chromatography (silica gel and ODS) and HPLC. *A. excelsa* yielded betulinic acid (1) as its antiproliferative component, while *A. flava* gave palmatine (2) and jatrorrhizine (3) as its antiplasmodial components. These compounds were identified based on the comparison of their spectral data [nuclear magnetic resonance (NMR) and mass spectroscopy (MS)] with literature values. The IC\(_{50}\) value of betulinic acid against A2780 cells was determined to be 20.6 μM. Both palmatine and jatrorrhizine had strong growth inhibitory activity against *P. falciparum* Dd2, with IC\(_{50}\) values of 0.41 μM and 0.43 μM, respectively.

INTRODUCTION

Ethnopharmacological studies have documented that many local communities in the Philippines are still using plants as medicines to cure various illnesses such as colds, fever, and stomach ache (Sia et al. 1998; Abe and Ohtani 2013). Many of these medicinal plants have been reported to possess various pharmacological activities such as antioxidant, anti-diarrheal, antiproliferative, antimicrobial, and lipid-lowering activities (Fuentes et al. 2010; Gorgonio and Fuentes 2011; Valle et al. 2015; Bautista et al. 2017). Efforts to validate the health benefits of medicinal plants have led to the approval of ten medicinal plants for traditional use by the Philippines’ Department of Health (Akarasereenont et al. 2015). Moreover, some of these Philippine plants have been reported to be sources of biologically active and novel compounds that could be potential leads for future drug development (Hernandez et al. 2008; Villaseñor and Sanchez 2009; Tan et al. 2014).

For many years, natural products – particularly from terrestrial plants – have been a valuable source of drug leads (Kinghorn et al. 2011), and many of the isolated compounds have been reported to be potential drugs against cancer and malaria (Fuentes et al. 2015; Du et al. 2017). The present status of cancer and malaria incidence is a major cause of concern (WHO 2010; Ferlay et al. 2018). Ovarian cancer is still one of the cancers with the highest incidence and mortality among females in the country (Bray et al. 2018; Ferlay et al. 2019). On the
other hand, even though there is an observed decrease in the number of malaria cases in the Philippines; there are still provinces that are considered malaria-endemic (Liu et al. 2013). Hence, tapping natural resources as possible sources of active compounds with anticancer and antimalarial activities remains an attractive approach to the discovery of therapeutic strategies to combat these diseases. In this study, we report the screening of some medicinal plants for their inhibitory activity against the A 2780 human ovarian cancer cell and Plasmodium falciparum, the parasite that causes malaria. A total of nineteen extracts from nine different plant species were investigated. Of these extracts, those of Alphitonia excelsa and Arcangelesia flava were studied further for their active compounds. Betulinic acid (I) was isolated from A. excelsa but showed only weak antiproliferative activity. A. flava provided palmatine (2) and jatrorrhizine (3), and both exhibited strong growth inhibitory activity against the Dd2 strain of P. falciparum. The present study provides additional information on the biological activities of the plant species found in Samar Island.

**MATERIALS AND METHODS**

**General Experimental Procedures**

NMR spectra were recorded on either Bruker Avance II (500 MHz) or Agilent M R 4 (400 MHz) spectrometers in either CDCl$_3$ or CD$_3$OD. High-resolution electrospray ionization mass spectra (HRESIMS) were obtained on an Agilent 6220 LC-TOF-MS in the positive ion mode. Column chromatography was conducted using silica gel PSQ100B.

**Plant Materials**

All plant samples were collected at Giporlos, Eastern Samar. The Department of Environment and Natural Resources (DENR) has issued Wildlife Gratuitous Permit (DENR GP No. RO8 2016-47) for the collection of plant materials. Some plant samples were identified by Mr. Jay Torrefiel of the Division of Natural Sciences and Mathematics. The collected plant samples were air-dried at room temperature until moisture content was below 5%. The two plants selected for detailed investigation were identified as Alphitonia excelsa (Fenzl) Reissek ex Endl. and Arcangelesia flava (L.) Merr. Voucher specimens of these two plants were submitted to the University of the Philippines Los Baños Natural History Museum and have the voucher numbers A1011 and Tu009.

**Extraction and Isolation**

The dried samples were cut into small pieces, macerated with methanol and allowed to stand overnight at room temperature. They were then filtered and the residue was extracted again with methanol twice and filtered. The filtrates were combined and evaporated to dryness in vacuo. The extraction used 13.7 g of A. excelsa twig samples and 22.3 g of A. flava stem samples. The extraction process provided 1.9% and 9.9% yield of crude extract, respectively. The crude extracts were then submitted to the Department of Chemistry, Virginia Tech, USA for screening for anticancer activity and to the University of Georgia for screening for antimalarial activity.

**Alphitonia excelsa.** The methanolic extract of A. excelsa (0.6 g) was resuspended in 10% methanol and partitioned between hexane, ethyl acetate (EtOAc), and water to provide hexane (32.0 mg), EtOAc (96.3 mg), and aqueous (387.4 mg) extracts. The extracts and subsequent fractions were then subjected to antiproliferative activity assay. The hexane extract (IC$_{50}$ = 9.5 µg/mL) was subjected to silica gel open chromatography ($φ$25 x 230 mm) using a hexane: EtOAc gradient (8:2 – 7:3) to give fractions 1A – 11. Bioactive fraction 1F (8.5 mg, viability = 11% at 20 µg/mL), which was eluted using hexane: EtOAc c (8:2), was subjected to solid phase extraction (HyperSip silica 2.8 mL) using DCM : MeOH (99:1 – 0:1) and gave Fractions 1F-1 to 1F-3. Fraction 1F-2, after crystallization, was identified as betulinic acid (1) (1.56 mg).

Betulinic acid (I): white crystalline solid; $^1$H (500 MHz, CDCl$_3$): 3.18 (1H, dd $\delta$ =11.4, 4.8 Hz, H-3), 0.69 (1H br d $\delta$ =9.2 Hz, H-5), 2.20 (1H td $\delta$ =3.4, 12.2 H-13), 2.26 (2H dt $\delta$ =2.9, 12.5 H-16), 2.99 (1H td $\delta$ =5.2, 10.8 H-19), 0.96 (3H s H$_3$-23), 0.75 (3H s H$_3$-24), 0.94 (3H s H$_3$-25), 0.82 (3H s H$_3$-26), 0.97 (3H s H$_3$-27), 4.74 (1H m H$_3$-29), 4.61 (1H s H$_2$-29), 1.69 (3H s H$_3$-30); $^{13}$C NMR (125 MHz, CDCl$_3$) 38.9 (C-1), 27.6 (C-2), 79.1 (C-3), 39.0 (C-4), 30.6 (C-5), 18.4 (C-6), 34.5 (C-7), 40.8 (C-8), 50.9 (C-9), 37.3 (C-10), 21.0 (C-11), 25.6 (C-12), 38.5 (C-13), 42.6 (C-14), 30.6 (C-15), 32.3 (C-16), 56.3 (C-17), 49.4 (C-18), 47.0 (C-19), 150.5 (C-20), 37.1 (C-21), 29.8 (C-22), 28.1 (C-23), 15.5 (C-24), 16.2 (C-25), 16.3 (C-26), 14.8 (C-27), nd (C-28), 109.9 (C-29), 19.5 (C-30); HRESIMS m/z 439.3609 [M-H$	ext{O}^+$]+, 457.3700 [M+H]+. Compound I was identified as betulinic acid (Dais et al. 2017).

**Arcangelesia flava.** The methanol extract of A. flava (108 mg) was subjected to polyamide column chromatography (using Pasteur pipet and methanol as eluant) to remove the tannins. The tannin-free extract was then partitioned between hexane, EtOAc c, and water to afford the hexane (7.2 mg), EtOAc c (16.3 mg), and aqueous (77.3 mg) extracts. The extracts and subsequent fractions were then subjected to antiproliferative activity assay. The bioactive aqueous fraction (IC$_{50}$ < 1.25 µg/mL) was subjected to silica column chromatography ($φ$25 x 210 mm) with elution with DCM : MeOH : MeCN (10: 3: 1 – 0.1% TFA in MeOH) to provide Fractions 1A – 1J. Bioactive fractions...
RESULTS AND DISCUSSION

A screening study of the plant collected from Giporlos, Eastern Samar was conducted for their growth inhibitory activity against the A2780 human ovarian cancer cell line and P. falciparum. A. excelsa (twigs) showed an IC_{50} value of 17.5 μg/mL, which was considered to be weakly active. The A. excelsa twig extracts were selected for isolation of its active constituent(s) because it was available in a sufficient amount. Its methanol extract was partitioned to provide hexane, EtOAc and aqueous extracts. The hexane extract, which showed the highest activity, was further isolated using column chromatography on silica gel followed by C18 solid-phase exchange chromatography. This isolation process yielded 1 (Figure 1) after crystallization. Data from NMR experiments and MS analysis revealed that 1 is betulinic acid. Compound 1 is an abundant labdane-type triterpenoid of many Alphitonia species (Dunstan et al. 1998; Raju et al. 2015). However, it exhibited only a weak growth inhibitory activity against A2780 cells (Table 1). Betulinic acid has been previously reported to have cytotoxicity against A2780 and various other cancer cell lines such as colorectal adenocarcinoma and melanoma cells (Zuco et al. 2002; Król et al. 2015). The study of Zuco et al. (2002) presented a lower IC_{50} (1.8 μg/mL), but the A2780 cancer cells were treated for 72 h as compared to the 48-h treatment used in this study.

There are only a few reports on the screening of Philippine plants for antimalarial activity. Using a mouse model, 28 different Philippine plants were screened...

**Figure 1.** Isolated compounds from A. excelsa (1) and A. flava (2 and 3).
Table 1. Antiproliferative and antiplasmodial activities of the isolated compounds from A. excelsa and A. flava.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Antiproliferative activity against the A2780 ovarian cancer cell line IC_{50} (μM)^c</th>
<th>Antiplasmodial activity against P. falciparum Dd2 strain IC_{50} (μM)^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.6 ± 10.8</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>NA</td>
<td>0.41 ± 0.05</td>
</tr>
<tr>
<td>3</td>
<td>NA</td>
<td>0.43 ± 0.05</td>
</tr>
</tbody>
</table>

^cData are presented as mean ± SD (n = 6), each independent experiment was conducted in triplicate, positive control: Taxol (IC_{50}: 21.9 ± 1.1 nM); NA - no activity observed at the highest concentration (20 μg/mL).

^bData are presented as mean ± 5SEM (n = 3), ND - not determined (estimated IC_{50} is greater than 5 μg/mL).

for their antimalarial activity and none of the screened plants was considered to be active (Popp et al. 1967). In another study, extracts of Blumea balsamifera and Citrus microcarpa were reported to have a potential antiplasmodial effect (Tongol-Rivera et al. 1996). In this investigation, 19 extracts were screened using a high-throughput screening assay that utilizes the quantitation of DNA of the parasitemia to determine the viability of P. falciparum Dd2 after treatment with either fractions or compounds. This study led to the identification of A. flava (stems) to have the most potent activity. In the Philippines, ethnopharmacological studies have not identified A. flava as a plant with antimalarial activity. Some Philippine locals have used this plant for the treatment of amenorrhea, stomach aches, and skin infections (Sia et al. 1998; UPM-NIH et al. 2012; Ong and Kim 2014). However, in Vietnam, A. flava is being used by their locals as an antimalarial agent, and stem extracts of A. flava have previously been reported to exhibit antimalarial activity (IC_{50} = 0.9 ± 0.1 μg/mL) (Nguyen-Pouplin et al. 2007). Their study, however, did not isolate the active compounds but implied that the activity exhibited by the extract is due to the action of berberine, palmatine, and jatrorrhizine, which are major compounds of this plant. It has been shown that berberine inhibits the telomerase activity of P. falciparum, which could be one mechanism of its antimalarial activity (Sriwilaijareon et al. 2002). In this study, activity-guided isolation on the A. flava stem extract was conducted for the identification of both known and potential new antimalarial compounds. The methanol extract of A. flava was partitioned between hexane, EtOAc, and water. The aqueous extract was the most active fraction, and bioassay-guided fractionation of this extract led to the isolation of the two alkaloids palmatine (2) and jatrorrhizine (3) (Figure 1). These two compounds both showed strong antiplasmodial activity with IC_{50} values of 0.41 and 0.43 μM, respectively (Table 1). Palmatine and jatrorrhizine have previously been reported to have antimalarial activity (Wright et al. 2000; Baghdikian et al. 2013). However, palmatine and jatrorrhizine exhibited weaker antiplasmodial activities against strain W2 (IC_{50} = 3.15 μM) (Baghdikian et al. 2013) and strain K1 (IC_{50} = 3.0 μM) (Wright et al. 2000) as compared to this study.

In conclusion, A. excelsa exhibited promising antiproliferative activity against the A2780 cell line during the screening activity. However, only betulinic acid was isolated and it exhibited weak antiproliferative activity against the A2780 cell line. Extracts of A. flava have previously been reported to have antimalarial activity, and the isolation of the alkaloids palmatine and jatrorrhizine confirms that these are the compounds responsible for this activity.

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REFERENCES


