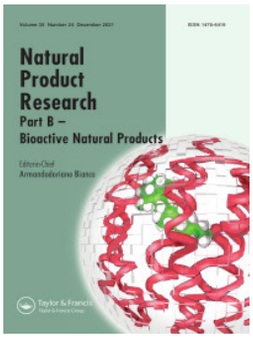

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SHORT COMMUNICATION

The anticancer activity of two glycosides from the leaves of *Leea aequata* L.

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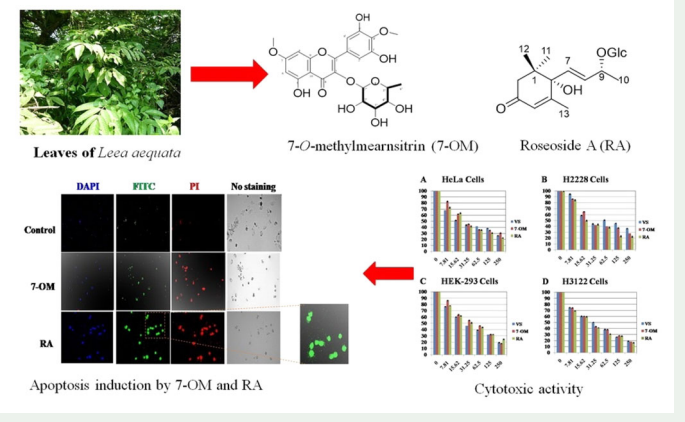
ABSTRACT

Two compounds (7-*O*-methylmearnsitrin (7-OM) and roseoside A (RA) were identified and characterized from the leaves of *Leea aequata* (*L. aequata*) L. The cytotoxicity of 7-OM and RA on HeLa cells was performed using MTT. The 7-OM and RA showed significant inhibition of HeLa cell proliferation with an IC_{50} of 22 and 20 μg/mL, respectively when compared with the standard vincristin sulphate (VS) (IC_{50} of 15 μg/mL). Moreover, the 7-OM and RA significantly inhibit other cancer cells (HEK-293, H228, and H3122) when compared with the VS and the cytotoxic activity of the compounds might show through the induction of apoptosis. Strikingly, annexin-V and PI signals could barely be detected in control cells, while strong fluorescence densities were observed in response to treatment indicating that these compounds have capacity to induce HeLa cell apoptosis. Our results suggest that the anticancer activity of 7-OM and RA was due to the induction of apoptosis.



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1. Introduction

Cancer is one of the leading causes of death globally with an increasing trend in developing countries. Globally 1 in 6 deaths is due to cancer and will be approximately twice by 2030 (Aggarwal et al. 2009). The WHO estimates that by 2040 over 29.5 million new cases of cancer will be diagnosed annually worldwide, with 16.5 million death annually (Bray et al. 2018; Shah et al. 2019). This alarming rate of cancer mortality can be reduced if it is diagnosed at an earlier stage and treated with proper medications. The currently used anticancer drugs have limited use due to their other serious health-related complications. Over 60% of existing anticancer agents are derived from natural sources, including plants, vegetables, fruits, spices, marine organisms, and microorganisms (Newman et al. 2002; Sharifi-Rad et al. 2019). Among these, plants have a long history of medicinal use in the treatment of cancer. Over the past 30 years, approximately 45% of all anticancer drugs have been derived directly or indirectly from plant compounds, in which 12% are natural products and 33% are semisynthetic derivatives of natural products (Cragg and Pezzuto 2016). Therefore, plants are a kind of natural gift to humans for protection and prevention from diseases, including cancer. Recently, our groups (Islam et al. 2013; Alam et al. 2016; Rahman et al. 2019) reported the anticancer activity of several plants in EAC (Ehrlich ascites carcinoma) cells-induced tumor bearing mice. These findings support the anticancer activity of plant species available in Bangladesh. *Leea aequata* (shrub, Family: Leeaceae) is a traditional medicinal plant distributed in India, Bangladesh, China, Malaysia, Philippines, and Vietnam (Chen and Wen 2007). The seeds, stems, tubers and roots of *L. aequata* are used in local medicine as anesthetics, anthelmintic, and antibacterial agents (Yusuf et al. 2009; Jain et al. 2010). However, limited studies have been conducted on the isolation and biological activity of this species (Tun et al. 2019), hence the plant was chosen for phytochemical investigation. Here, two compounds from ethyl acetate fraction (EAF) of *L. aequata* were purified and characterized as 7-*O*-methylmearnsitrin (7-OM) and roseoside A (RA), and the compounds showed significant anticancer activity in multiple human cancer cell lines. Strikingly, the anticancer activity was due to induction of apoptosis.

2. Results and discussion

The leaves of *L. aequata* were extracted with 80% methanol to get the crude methanolic extract (CME). The CME was successively fractioned with petroleum ether, chloroform, ethyl acetate, and finally with water to get four fractions: petroleum ether fraction (PEF), chloroform fraction (CHF), ethyl acetate fraction (EAF) and aqueous fraction (AQF), respectively. Qualitative phytochemical screening of these fractions revealed the presence of flavonoids, phenolics, saponins, tannins, steroids, glycosides, and alkaloids (Supplementry material, Table S1). Based on phytochemical screening and TLC behavior of the extractives, the EAF was subjected to column chromatography (CC) with different solvent systems (Supplementry material, Figure S1, Table S2) and several fractions (Fr.1-Fr.25) were obtained (Supplementry material, Table S3). The fractions (Fr.8-Fr.10) showed identical three spots (named as JM1, JM2, and JM3) (Supplementry material, Figure S2), which were pooled together (known as fr-2) and

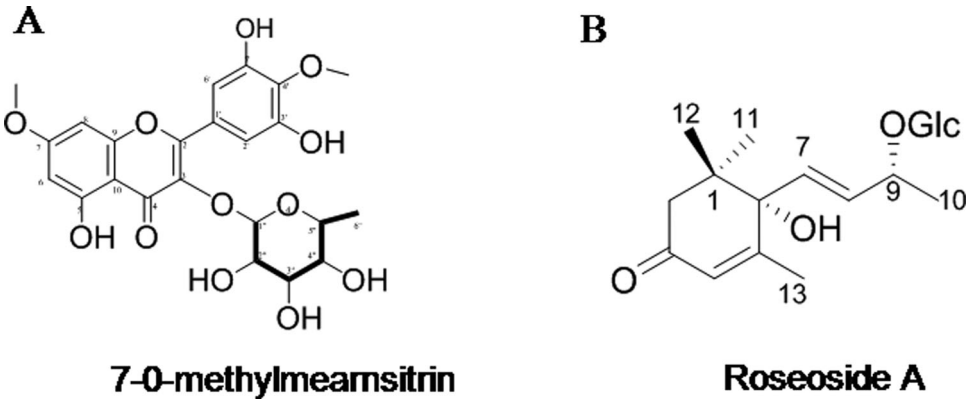

Figure 1. Structure of compounds. (A) 7-*O*-methylmearnsitrin and (B) Roseoside A.

subjected to preparative thin layer chromatography (PTLC) (20 × 20 cm) and the bands were scrapped off from the PTLC plates (Supplementry material, Figure S3) to afford three compounds (Supplementry material, Figure S4). Interestingly, JM1 was characterized as 7-*O*-methylmearnsitrin (7-OM) (Figure 1(A)) using ^{1}H and ^{13}C-NMR, 2 D-NMR, mass data (Supplementry material, Table S4, Figure S(5A-5E), Figure S7); on the contrary, JM2 was characterized as Roseoside-A (RA) (Figure 1(B)) using ^{1}H and ^{13}C-NMR, 2 D-NMR, mass spectrometry data (Supplementry material, Table S5, Figure S(6A-6D), Figure S8), and previous published data (Takeda et al. 1997; Chung et al. 2004). JM3 was a mixture of compounds and the elucidation of the structure is in progress.

To assess the cytotoxicity of the 7-OM and RA, cell proliferation assay was performed using MTT. HeLa cells were treated with different concentration (7.81–250 μg/mL) of the tested samples (7-OM and RA). Interestingly, 7-OM and RA showed strong dose-dependent inhibition of HeLa cells proliferation with an IC_{50} of 22 and 20 μg/mL, respectively, which were closely resemble to that of the standard, VS (IC_{50} of 15 μg/mL) (Supplementry material, Figure S9A). This study confirmed that the cytotoxic activity of the compounds is considerably effective, likely to the result reported by Ito et al in 2002.

Next, whether these compounds have cytotoxic activity on other cell lines (HEK-293, H3122, and H2228) or not, the cell proliferation assay was further performed using MTT. Strikingly, the 7-OM and RA exhibited significant dose-dependent inhibition on H2228 (Supplementry material, Figure S9B), HEK-293 (Supplementry material, Figure S9C) and H3122 cells (Supplementry material, Figure S9D) suggesting that these compounds showed differential functions against several types of cancer. These results are consistent with those of the data published previously (Ito et al. 2002; Chung et al. 2004; Matsunami et al. 2010).

Furthermore, the effect of the 7-OM and RA on HeLa cell apoptosis was evaluated using DAPI, Annexin-V FITC, and PI triple florescence staining. DAPI is used as a marker of cell membrane permeability seen in very late apoptotic cells; whereas, Annexin-V FITC staining can identify apoptosis at an earlier stage (Miao et al. 2013). In this study, DAPI, Annexin-V FITC, and PI signals could barely be detected in control cells (without treatment), while strong fluorescence densities were observed in response to treatment indicating that these compounds have capacity to induce HeLa cell apoptosis (Supplementry material, Figure S10) through early and late stages.

Antitumor activity of roseoside A (RA) was reported previously (Ito et al. 2002), however, to our knowledge this is the first report on cytotoxic activity of 7-*O*-methylmearnsitrin (7-OM).

3. Conclusions

In this study, we have characterized two compounds as 7-*O*-methylmearnsitrin (7-OM) and roseoside A (RA) from the leaves of *L. aequata*., This is the first report of their occurrence in the plant of *L. aequata*. Both the compounds showed strong anticancer activity in different cancer cells and their activities were due to the induction of apoptosis. To the best of our knowledge cytotoxic activity of 7-*O*-methylmearnsitrin (7-OM) has not been yet investigated and is the first report. Further study is necessary to find out the exact mechanism of their anticancer activity at molecular level.

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Disclosure statement

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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