SHORT REPORT

A C G publications

Rec. Nat. Prod. X:X (2020) XX-XX

records of natural products

Cytotoxic Activity and Phytochemical Constituents of *Macrosolen bidoupensis* Tangane & V.S. Dang

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(Received February 08, 2020; Revised September 01, 2020; Accepted September 02, 2020)

Abstract: Phytochemical investigations of the whole plants of *Macrosolen bidoupensis* Tangane & V.S. Dang, a new species discovered in Viet Nam were conducted and led to the purification of ten compounds, comprising three lupane-type (**1-3**), three friedelane-type (**4-6**), and one glutinane-type (**7**) triterpenoids and three cholestane-type steroids (**8-10**) using various chromatographic methods. Their structures were characterized by HR-ESI-MS, NMR experiments and comparison with previous literature. Compounds **1**, **3** and **5** expressed moderate cytotoxicity against two tested cell lines - MDA-MB-231 and RD (IC_{50} ranged from 34.19 to 74.25 μM), whereas compounds **4** and **6** exhibited selective cytotoxic activity (IC_{50} ranged from 29.07 to 45.20 μM).

Keywords: *Macrosolen bidoupensis*; Loranthaceae; cytotoxic activity; triterpenoid; steroid.

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products Month-Month 2020 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.197.20.02.1545

1. Plant Source

The whole plants of *Macrosolen bidoupesis* were collected in Bidoup Nui Ba National Park, Lam Dong Province, Viet Nam in December 2018 and identified by Dr. Van Son Dang, Institute of Tropical Biology, Vietnam Academy of Science and Technology. A voucher specimen (No.VH/PHAT-MB1218) was deposited in Bioactive Compounds Laboratory, Institute of Chemical Technology, Vietnam Academy of Science and Technology.

2. Previous Studies

Macrosolen bidoupensis is a new species that was discovered in Bidoup, Nui Ba National Park, Lam Dong Province, Viet Nam in 2017 [1]. There is no report on the phytochemical components and biological activities of this species.

3. Present Study

The whole-plant powder of *M. bidoupensis* (8 kg) was extracted with 96% EtOH (3 x 30 L) at room temperature. After filtering the residue, the solvents were removed under low pressure and the crude extract was obtained. The crude extract (1000 g) was applied to liquid-liquid extraction procedures and successively partitioned into *n*-hexane, ethyl acetate (EtOAc), and aqueous partition. The *n*-hexane extract (98 g) was subjected to silica gel column chromatography with mobile phase *n*-hexane-EtOAc (100-0 to 0-100, v/v) to yield five fractions (MBH.I-MBH.V). As the same manner, the EtOAc extract (33 g) was separated on silica gel column chromatography with solvent *n*-hexane-EtOAc gradient to obtain five fractions (MBE.I-MBE.V).

MTT assay was performed using the MTT test, as previously described [2]. For the cytotoxicity, the percentage of control (%) was calculated = $OD_{570\ sample}/OD_{570\ control}$ x 100% measured at the different concentrations (12.5, 25.0, 50.0 and 100.0 μg/mL) by MTT assay.

The cytotoxic effects of all fractions from the *n*-hexane and ethyl acetate extracts of *Macrosolen bidoupensis* whole plants were evaluated against two human cancer cell lines (MDA-MB-231 and RD) by MTT assay (Table 1). All of the fractions except fraction MBE.V displayed cytotoxicity (IC_{50} ranged from 4.52 ± 0.33 to 68.66 ± 1.81 μg/mL). Among them, fractions MBH.III and MBE.II revealed strong cytotoxic properties towards breast cancer cell lines (MDA-MB-231) with IC_{50} value of 4.52 ± 0.33 and 4.75 ± 0.42 μg/mL, respectively, while fractions MBH.IV and MBE.III exhibited significant cytotoxic activity against Rhabdomyosarcoma cell lines (RD) with IC_{50} value of 20.37 ± 1.01 and 19.78 ± 3.12 μg/mL, respectively. Therefore, all positive fractions were further investigated for their phytochemical constituents.

Fraction MBH.I (45 g) was eluted with solvent *n*-hexane-EtOAc gradient (100-0 to 0-100, v/v) on silica gel column to give six sub-fractions (MBH.I.1-MBH.I.6). Subfraction MBH.I.2 (7 g) was chromatographed with solvent system *n*-hexane-EtOAc (99:1, v/v) to obtain compounds **4** (900 mg) and **7** (5 mg). Fraction MBH.II (39 g) was eluted with solvent *n*-hexane-EtOAc gradient (100-0 to 80-20, v/v) on silica gel column to give six sub-fractions (MBH.II.1-MBH.II.6). Subfraction MBH.II.2 (10 g) was chromatographed with solvent system *n*-hexane-EtOAc (99:1 to 95:5, v/v) to obtain compounds **1** (1000 mg), **2** (6 mg), **8** (4 mg), **9** and **10** (50 mg). Fraction MBH.III (3 g) was separated using solvent *n*-hexane-EtOAc (98-2, v/v) to give **1** (50 mg) and **3** (6 mg). Fraction MBE.II (8 g) was rechromatographed on silica gel with solvent *n*-hexane-EtOAc gradient (99-1 to 80-20, v/v) to yield **5** (20 mg) and **6** (7 mg).

The HR-ESI-MS and NMR data of the three others were consistent with those reports in the literature for lupeol **(1)** [3,4], betulin **(2)** [5], betulinic acid **(3)** [6], friedelin (**4**), 3α-friedelanol (**5**), 3β-hydroxyfriedelane-28-oic acid (**6**) [7], 3β-hydroxyglutin-5-ene (**7**) [8], 3β-cholesterol acetate (**8**) [9], and a mixtures of 24β-ethylcholesterol (**9**) and 24β-ethylcholesta-5,22-diene-3β-ol (**10**) [10] (Figure 1). All of those compounds, except **1,** were reported from the genus *Macrosolen* for the first time.

Table 1. Cytotoxic activity of all fractions

Cell line	Fraction	IC_{50} (μg/mL)
MDA-MB-231	H.I[a]	-
	H.II	31.55 ± 1.51
	H.III	4.52 ± 0.33
	H.IV	15.05 ± 0.99
	H.V	68.66 ± 1.81
	E.I	27.24 ± 1.00
	E.II	4.75 ± 0.42
	E.III	18.39 ± 2.15
	E.IV	42.96 ± 1.46
	E.V	> 100.00
	Paclitaxel	8.38 ± 0.75 (μM)
RD	H.I[a]	-
	H.II	32.30 ± 0.34
	H.III	48.40 ± 3.05
	H.IV	20.37 ± 1.01
	H.V	41.36 ± 0.62
	E.I	41.94 ± 3.56
	E.II	30.12 ± 2.96
	E.III	19.78 ± 3.12
	E.IV	50.43 ± 1.11
	E.V	> 100.00
	Paclitaxel	5.73 ± 0.27(μM)

[a]insoluble

The cytotoxic activities of five triterpenoids (**1**, **3**-**6**) were examined against two human cancer cell lines (MDA-MB-231 and RD) by MTT assay (Table 2) and compounds **1**, **3** and **5** showed meaningful cytotoxicity against all tested cell lines (IC_{50} ranged from 34.19 ± 0.53 to 74.25 ± 0.46 μM), whereas, compound **4** displayed selective cytotoxic property against MDA-MB-231 cells (IC_{50} value of 45.20 ± 1.37 μM) and compound **6** exhibited cytotoxic effect against RD cells (IC_{50} value of 29.07 ± 3.47 μM). According to those results, we conlclude that the friedelane and lupane triterpenoids evinced as the essential components that accounted for the cytotoxic effect of *M. bidoupensis* against two tested human cancer cell lines. However, further clinical examinations are required to determine the molecular mechanisms of cytotoxicity as well as qualitative and quantitative identification of main biological triterpenoid markers (**1** and **4**) from this species.

Table 2. Cytotoxic activity of five triterpenoids **1**, **4**-**6**

Cell line	Compounds	IC_{50} (μM)
MDA-MB-231	**1**	57.78 ± 1.95
	3	34.19 ± 0.53
	4[b]	45.20 ± 1.37
	5	51.78 ± 0.73
	6[b]	> 50
	Paclitaxel	8.38 ± 0.75
RD	**1**	74.25 ± 0.46
	3	34.41 ± 1.57
	4[b]	> 50
	5	70.95 ± 1.94
	6[b]	29.07 ± 3.47
	Paclitaxel	5.73 ± 0.27

[b]the maximal soluble concentration is 50 μM

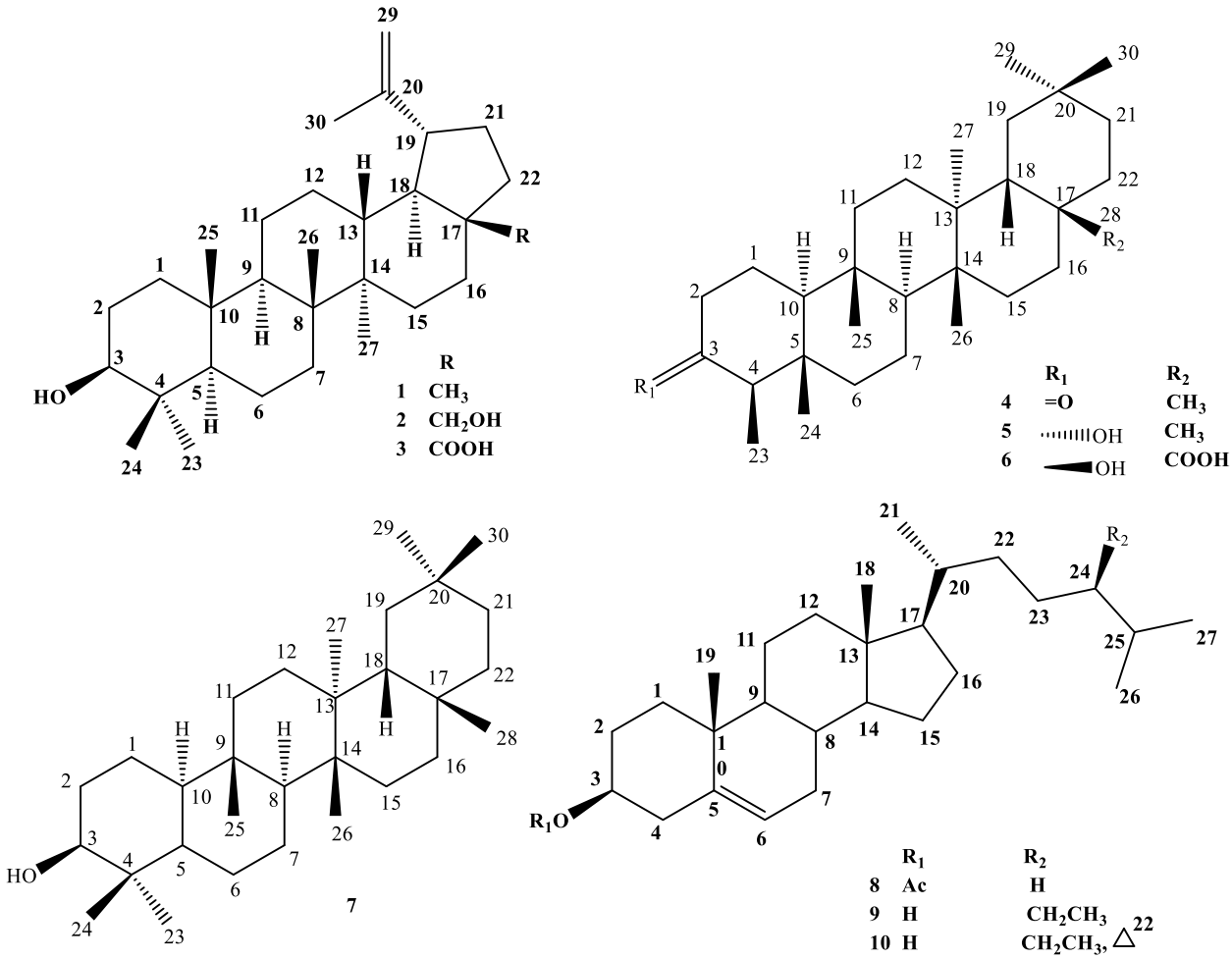

Figure 1. Chemical structures of compounds (**1**-**10**)

In conclusion, a systematic chemical investigation of *Macrosolen bidoupensis* Tangane & V.S. Dang, was reported for the first time herein. Phytochemical investigation of the active extracts indicated that the triterpenoids present in this species accounted for their cytotoxic activity.

Acknowledgments

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.01-2018.48.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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