SHORT REPORT

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Isolation, Identification and Antiproliferative Activity of Triterpenes from the Genus *Monotheca* A. DC.

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Abstract: The *Monotheca* A. DC. is a monotypic genus of the family Sapotaceae, which is widely distributed in Afghanistan, Djibouti, Northern Somalia, Oman, Pakistan and Southern Ethiopia. North-west Pakistan is the main region where *Monotheca buxifoli*a (Falc.) A. DC., the only species of this genus, locally known as "Gurgura", grows abundantly. It is an evergreen, fruit-producing medicinal tree. Bioassay-guided fractionation of the aerial parts of *M. buxifolia* afforded lupeol **(1),** lupeol acetate **(2)**, betulin **(3)**, oleanolic acid **(4)** and *β*-amyrin **(5)** from the *n*-hexane and the chloroform fractions. This is the first report on the isolation, and identification of triterpenes **(1-5)** as the major compounds in the active fractions with antiproliferative property, and also on the antiproliferative activity of *M. buxifolia* extract and fractions against the human lung cancer cell line NCI-H460 *in vitro*.

Keywords: *Monotheca buxifolia*; Sapotaceae; antiproliferative property; triterpene; sulforhodamine B assay; lung cancer. © 2016 ACG Publications. All rights reserved.

1. Plant Source

In continuation of our studies on medicinal plants from the Pakistani flora [1-3], *Monotheca buxifolia* (Falc.) A. DC. has been studied. In this short report, the isolation and identification of triterpenes **(1-5)** as the major compounds in the active fractions with antiproliferative property, and also the antiproliferative activity of *M. buxifolia* extract and fractions against the human lung cancer cell line NCI-H460 *in vitro* (Table 1) have been reported for the first time.

The aerial parts of *M. buxifolia* (Falc.) A. DC. were collected during April 2011 from the Swat region in Pakistan. A voucher specimen representing this collection (GC Bot. Herb. 815) has been deposited in the Dr. Sultan Ahmed Herbarium, Department of Botany, GC University, Lahore, Pakistan.

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2. Previous Studies

Previous studies on this plant have demonstrated its antioxidant and antimicrobial properties [4, 5]. Whilst a report on the constituents of unsaponifiable fraction of the seed oils of *M. buxifolia* is available [6], to the best of our knowledge, there is no report on the isolation and identification of triterpenes from the aerial parts of this plant, as well as on the assessment of antiproliferative activity of the extracts, fractions and these compounds published to date.

3. Present Study

Shade-dried, ground aerial parts of *M. buxifolia* (4.2 kg) were macerated in methanol (MeOH, 3 x 7 L) at room temperature and the pooled extract was concentrated by rotary evaporator under reduced pressure to yield 600 g of a gummy mass. This residue was suspended in water (400 mL) and successively partitioned with *n*-hexane (4 x 400 mL), chloroform (4 x 400 mL) and ethyl acetate (EtOAc, 4 x 400 mL) to obtain 150, 100 and 150 g of respective fractions. The *n*-hexane fraction was subjected to vacuum liquid chromatography (VLC) over silica gel using *n*-hexane-EtOAc as the solvent system in increasing order of polarity. The hexane sub-fraction 2, collected with *n*-hexane:EtOAc (7:3) was re-chromatographed and eluted with *n*-hexane:EtOAc (7:3) to isolate compound **1** (10 mg). The sub-fraction 3, which was obtained with *n*-hexane:EtOAc (3:2), was re-chromatographed over silica gel and eluted with *n*-hexane:EtOAc (3:2) to isolate compound **2** (8 mg). Similar chromatographic purification of the chloroform fraction afforded three other triterpenes **(3-5)** (Figure 1). Briefly, the chloroform fraction was loaded on silica gel column and eluted with *n*-hexane-chloroform on increasing polarity resulting in three major sub-fractions 1-3. Fraction 1, which was eluted with *n*-hexane-chloroform (7:3) was further purified by column chromatography using *n*-hexane-dichloromethane (1:1) as an eluent to afford compound **3** (13 mg). Sub-fraction 2, obtained with *n*-hexane-chloroform (6:4), was re-chromatographed over silica gel and eluted with *n*-hexane: dichloromethane (1:1) to afford compound **4** (30 mg). Sub-fraction 3, obtained from chloroform-MeOH (19:1) solvent system, was purified by column chromatography using dichloromethane-MeOH (9:1) as an eluent to yield compound **5** (15 mg). Structures of the isolated compounds **(1-5)** were conclusively determined by spectroscopic analyses, mainly MS, and 1D and 2D NMR spectroscopy as well as by comparing their spectroscopic data with respective published literature data [7-11].

*Lupeol (**1**)*: White amorphous solid; melting point 215°C; EI-MS, HREI-MS ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data were in agreement with the published data [7].

*Lupeol acetate (**2**):* White greasy substance; melting point 214-216°C; EI-MS, HREI-MS ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data were in agreement with the published data [8].

*Betulin (**3**):* White amorphous powder; melting point 217-219°C; EI-MS, HREI-MS ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data were in agreement with the published data [9].

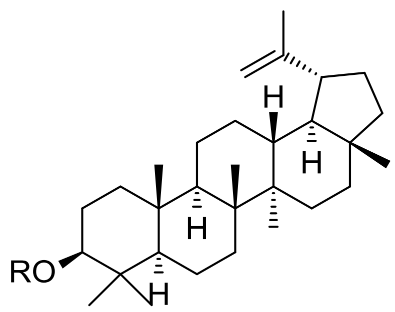
*Oleanolic acid (**4**):* White powder; melting point 304-306°C; EI-MS, HREI-MS ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data were in agreement with the published data [10].

*β-Amyrin (**5**):* White amorphous solid; melting point 196-198°C; EI-MS, HREI-MS ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data were in agreement with the published data [11].

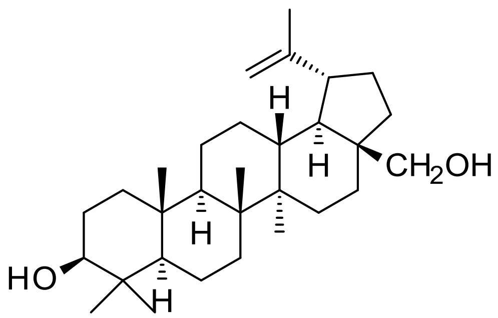
Whilst this is the first report on the occurrence of the triterpenes (**1-5**) in the genus *Monotheca*, other genera of the family Sapotaceae have been reported to produce these compounds [12-14].

Antiproliferative activity assay: The *in vitro* antiproliferative activity of the extract, fractions and isolated triterpenes (**1-5**) from *M. buxifolia* was assessed against the human lung cancer cell line NCI-H460 using the sulforhodamine B assay [15]. The cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium planted at a density of 5000 cell/well. Cells were washed with phosphate buffer saline (PBS) and detached with 0.025% trypsin-EDTA. The cell suspension was made by adding 10 mL of RPMI 1640 solution and centrifuged for 10 min. Cells viability was counted in a haemocytometer, which exceeded 90% through trypan blue exclusion. Optimal seeding density of cell suspension was made after dilution and 100 μL of cell suspension was poured into 96-well plate, incubated at 37°C. The test extract (MeOH) and fractions (*n*-hexane, chloroform and EtOAc) were initially dissolved in DMSO and passed through a 0.2 μm filter. Five concentrations (1.0, 10, 50,100, 250 μg/ mL) of all test samples were made by dilution in medium to determine the IC_{50} value. Similarly, five concentrations (0.1, 1.0, 5.0, 10, 50 μg/ mL) of each isolated triterpenes (**1-5**) were made along with the standard drugs doxorubicin and vinblastine as positive controls. Each concentration (100 μL) was added to well plates after 24 h of incubation. Cell growth was analyzed at the end of 72 h. After shaking plates for 20 min on a plate shaker, optical density was read at 570 nm. Percentage absorbance of test sample plates was compared to that of the control (non-treated cells) to investigate cell viability. The IC_{50} value of each test sample was calculated using the software WinNonLin Professional, Version 5.0.1.

In the sulforhodamine B assay, amongst the test fractions, the *n*-hexane and the chloroform fractions showed the highest degree of activity with IC_{50} values of 59.2 and 46.1 μg/mL, respectively, and were subjected to further chromatographic analyses. All isolated triterpenes **(1-5)** exhibited considerable antiproliferative activity. Among them, oleanolic acid **(5)** was the most active with an IC_{50} value of 6.80 ± 1.73 μg/mL, which compares favourably to both positive controls, doxorubicin (IC_{50} = 10.5 ± 1.4 μg/mL) and vinblastine (IC_{50} = 8.13 ± 1.0 μg/mL) (Table 2). Lupeol **(1)**, lupeol acetate **(2)**, betulin **(3)** and *β*-amyrin **(4)** had the IC_{50} values, 19.2 ± 4.0, 63.4 ± 1.0, 79.2 ± 4.0 and 58.7 ± 2.0, respectively (Table 1). To the best of our knowledge, this is the first report on the antiproliferative activity of *M. buxifolia.*



1 R = H
2 R = Ac



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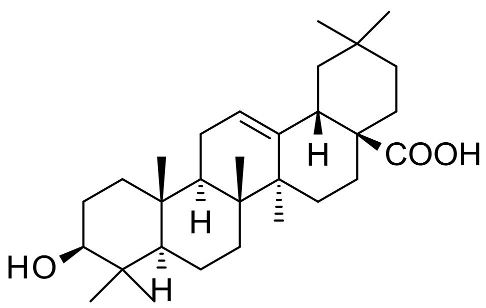

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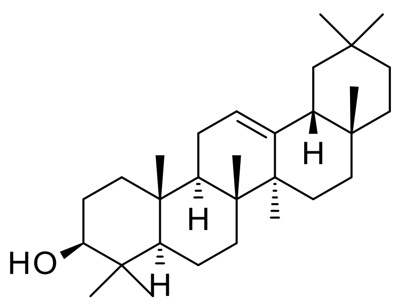

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Figure 1. Structures of triterpenes from *M. buxifolia* with antiproliferative property

This finding is in agreement with the previously reported potent antitumour activities of various triterpenes including lupeol (**1**), betulin (**3**) and oleanolic acid (**5**) [16-19]. The pentacyclic triterpene oleanolic acid (**5**) possesses a variety of pharmacological activities. Extensive studies on the synthesis of oleanolic acid (**5**) and its derivatives, and subsequent assessment of their anticancer potential have revealed their potential against various cancer cell lines [20]. Thus, the antiproliferative activity of the fractions was mainly owing to the presence of these well known bioactive compounds

(**1-5**). It was suggested that the antiproliferative activity of these triterpenes might be linked to their ability to directly inhibit tumour growth cell cycle progression and induce apoptotic cell death by triggering the mitochondrial pathway of apoptosis [21]. Antiproliferative activities of triterpenes are also generally attributed to their complementary effects [22]. In Japan, oleanolic acid (**5**) is recommended for the treatment of skin cancer therapy [23]. Cosmetics preparation and medicinal formulations are patented comprising of oleanolic acid as active ingredient for topical use to treat skin cancer and oral use for curing non-lymphatic leukemia [24, 25]. Long term use of oleanolic acid >3 months confirmed its safety for use, and a negligible side effects. Oleanolic acid has been used in health drinks and marketed in China for the treatment of liver disorders [26, 27].

In the present study, the elucidation of the antiproliferative activity of the MeOH extract and various fractions of *M. buxifolia*, and subsequent identification of bioactive compounds present in the active fractions and assessment of their antiproliferative potential have certainly identified this medicinal plant as a major source of antiproliferative compounds which could possibly be exploited commercially.

Statistical analysis: Data were expressed as means ± standard error of the mean (SEM). The graph was plotted using non-linear regression with the use of GraphPad Prism version 6.0 for Windows (GraphPad Software, San Diego, CA, USA).

Table 1. IC_{50} values of the extract, fractions and triterpenes from *M. buxifolia* against the human lung cancer cell line NCI-H460

Test samples	IC_{50} values (μg/mL)
Methanol extract	172.0 ± 4.1
n-Hexane fraction	59.2 ± 4.0
Chloroform fraction	46.1 ± 2.0
Ethyl acetate fraction	187.0 ± 1.1
Lupeol **(1)**	63.4 ± 1.0
Lupeol acetate **(2)**	80.4 ± 1.0
Betulin **(3)**	79.2 ± 4.0
β-amyrin **(4)**	58.7 ± 2.0
Oleanolic acid **(5)**	6.8 ± 1.73
Doxorubicin	10.5 ± 1.4
Vinblastine	8.1 ± 1.0

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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