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Research Article

Anticancer activity studies of root extract of *Aloe pirottae* A. Berger Endemic plant species of Ethiopia

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Abstract

In an effort to find new anticancer agents from natural products, crude methanol extract and four organic solvent fractions, n-hexane (HxF), chloroform(CHF), ethyl acetate(EAF) and n-butanol (BuF), from *Aloe pirottea* root, an endemic medicinal plant of Ethiopia, were investigated in vitro for their activities against A549, A2780, MIA-PaCa-2 and SNU-638 cancer cell lines at different concentrations including 0.1, 0.3, 1.0, 3.0, 10.0, and 30.0 µg/ml to determine the percentage of growth inhibition and IC_{50}values using the sulforhodamine B (SRB) assay. The results were compared to standard anticancer drug Etoposide. Results demonstrated that all extracts exhibited anticancer activity with different degrees of potency. HxF, CHF and EAF of *Aloe pirottea* root extract showed good cytotoxic activity against A549, A2780 and SNU638 with IC_{50} value ranging from 6.37 to 29.69 µg/ml and, except for CHF of *Aloe pirottea* root with IC_{50} value of 18.86 µg/ml, all exhibited no or weak cytotoxic activity against MIA-PaCa-2 with IC_{50} value of > 30.0 µg/ml. The highest cytotoxicity was found in the chloroform fraction. The responsiveness of solvent fraction to cell decreased as Etop>CHF > EAF > MeOH > HxF > BuF compared to reference standard anticancer drug Etoposide. The responsiveness of cell line to extracts were decreased as SNU-638 >A-549 > A2780 > MIA-PaCa-2. It was found that the percentage growth inhibition increases with increasing concentration. This finding shows that the extracted fractions from this plant species can be used as a potential source for producing anticancer drugs.

Keywords: *Aloe pirottea*, anticancer, Endemic, Etoposide, Cytotoxic activity

1. Introduction

Cancer continues to be a great threat to human life. Despite the tremendous progress in human medicine, cancer diseases are one of the major causes of death in humans worldwide. Lung cancer is the most common cause of cancer-related deaths in men and second most common in women after breast cancer[1].Of the two major classes of lung cancer, non-small cell lung cancer accounts for 75 % to 80 % and small cell lung cancer (SCLC) accounts for 15 % to 25 % of all lung cancer cases[2, 3]. Ovarian cancer (A2780) is the seventh-most common cancer among women and the eighth-most common cause of death from cancer. Pancreatic cancer (MIA-Paca-2) is the seventh highest and responsible for 6% of cancer deaths each year[4]. Globally, stomach cancer (SNU-638) is the fifth leading cause of cancer and the third leading cause of death from cancer making up 7% of cases and 9% of deaths. Global research studies revealed the necessity of alternative solution towards cancer because of increasing incidence of cancer death, lacking of effective therapies, emerging of chemotherapeutic drug resistance and side effects from chemotherapy like microsites, bone marrow toxicity, cardiotoxicity, pulmonary, renal and neurotoxicity[5]. The natural drugs derived from natural resources including plants, marine organisms and microorganisms and their derivatives account for over 60% anticancer agents[6]. Medicinal plants become an alternate solution to develop new and effec-

tive anticancer drugs with mild and /or no side effects, by methodical and scientific exploration of enormous pool of natural products[7]. In this study, endemic medicinal plant species *Aloe pirottea root* was selected based on literature and local healers. This plant with a history of multiple traditional uses was not studied for anticancer activity. *Aloe pirottae* A. Berger belongs to the family Aloaceae[8] and the leaf gel and sap are traditionally used as a folk medicine for the treatment of inflammation, viral, bacterial, fungal infections, malaria, tropical ulcer, gastrointestinal parasites, gallstone, eye diseases, constipation, burns, dermatitis and snake bite. Gel extracts are used for cleaning human colon and leaf latex as insect repellent[9]. Our objective is to carry *in vitro* studies of crude methanol extract and the organic solvent fractions (n-HxF, CHF, EAF and n-BuF) of root extract of *Aloe pirottea* plant species to demonstrate significant cytotoxicity against A549, A2780, MIA-PaCa-2 and SNU-638 cancer cell lines using the SNB assay.

2. Materials and Methods

The commercially available standard anticancer agent used in this study was Etoposide obtained from US Biological Swampscott, MA. All solvents used for preparation of the extracts were HPLC-grade and obtained from Korea Research Institute of Chemical Technology (KRICT) South Korea.

2.1 Plant collection and identification

Organic, naturally grown endemic medicinal plant *Aloe pirottae* (Figure 1) roots were collected from Addis Ababa, Oromia region, Ethiopia in November 2017. The plant was identified and authenticated by a botanist, Prof. Teshome Soromesa of the Biology Department, Addis Ababa University. The voucher specimen (Voucher No. A004/2017) was deposited in the herbarium of Biology Department, Addis Ababa University, Ethiopia.

2.2 Preparation of plant extract

The roots of plant were cleaned and cut into small pieces and air-dried in darkness under shade. Shade-dried plant material was ground to fine powder and was soaked in methanol for 3 days (X 3) three times and shaken ten times daily. Methanol was used in the ratio of 3 ml for 1 g of dry powdered plant material. After 3 days, methanol was squeezed out and filtered. The three filtrates were combined and concentrated to dryness in a rotary evaporator under reduced pressure at below 34°C. This solid or semisolid material is called the crude methanol extract (MeOHEx). The MeOHEx was suspended in distilled water in the ratio of 1 g extract in 10 ml water. This water suspension in separating funnel was extracted each for 12 hours three times (X 3) with the water immiscible organic solvent in increasing polarity (n-hexane, chloroform, ethyl acetate and n-butanol) and shaken in 30-minute interval. Each time, the volume of the organic solvent used was the same as that of the water layer. The organic layer was dried in a rotary evaporator, and the aqueous layer was extracted with the other organic solvent in the order of increasing polarity. Each time, the collected organic solvent layer was combined and evaporated by rotary evaporator to dryness at temperature between 30-33^{0}C, and the small amounts of wet extracts from each solvent were then lyophilized by using vacuum dryer.

2.3 Anticancer activity studies

The cancer cell lines including the human non-small cell lung cancer cell lines (A549), ovarian cancer cell lines (A2780), pancreatic cancer cell lines (MIA-Paca-2) and stomach cancer cell lines (SNU-638) were used for evaluation of anticancer activity. All cell lines were maintained using RPMI1640 cell growth medium (Gibco, Carlsbad, CA), supplemented with 5% fetal bovine serum (FBS) [Gibco], and grown at 37 °C in a humidified atmosphere containing 5% CO_2. The cytotoxicity of the crude methanol extract and its organic solvent fractions of the plant against cultured human tumor cell lines was evaluated on the already developed sulforhodamine B (SRB) method[10] at different concentrations of 0.1, 0.3, 1.0, 3.0, 10.0, and 30 µg/ml to determine percentage of growth inhibition and the IC_{50} (50% growth inhibition). Each tumor cell line was inoculated over standard 96-well flat-bottom micro plates and then incubated for 24 h at 37 °C in a humidified atmosphere of 5% CO_2. The attached cells were then incubated with serially diluted each samples. After continuous exposure to the compounds for 72 hour, the culture medium was removed from each well and the cells were fixed with 10% cold trichloroacetic acid at 4°C for 1 h. After washing with tap water, the cells were stained with 0.4% SRB dye and incubated for 30 min at room temperature. The cells were washed again and then solubilized with 10 mM buffered Tris base solution of pH 10.5. The absorbance

was measured spectrophotometrically at 520 nm with a micro titer plate reader. Each experiment was conducted in triplicate. The IC_{50} values of compounds were calculated by the nonlinear regression analysis. The IC_{50} was expressed as the concentration of drug reducing the plaque number by 50% as compared to mock-treated controls. It was calculated from a dose–response line obtained by plotting the percentage plaque reduction, with respect to the control plaque count, versus the logarithm of compound dose. Triplicate wells were utilized for each drug concentration tested.

3. Results and Discussion

As far as we know, the present work constitutes the first report studying the anti- proliferation potential of *A. pirottea* root against A459, A2780, MIA-PaCa-2 and SNU 638 cancer cells. As per SRB assay revealed, the plant root extract exhibited a broad spectrum in anti-cancer activity (Figure 2a-e). It revealed that extract and solvent fractions of plant induced dose-dependent cell growth inhibition effects in all the cell lines tested. The percentage of growth inhibition was found to be increasing with increasing concentration of test compounds. Three fractions showed strong to moderate cytotoxicity against A549 cells in culture with IC_{50} values ranging from17.06 to 29.89 µg/ml. Two fractions moderate cytotoxicity against A2780 cells in culture with IC_{50} values of 21.70 and 25.67 µg/ml. Three fractions strong cytotoxicity against SNU 638 cells in culture with IC_{50} values of 12.47, 12.88 and 14.69 µg/ml. CHF showed strong cytotoxicity against MIA-PaCa-2 cells in culture with IC_{50} values of 18.96 µg/ml. The fact that *A. pirottea* root extracts exhibited strong to moderate anticancer activity against all the cancer cell lines tested. This result may reflect that ethnopharmacological knowledge and texts of traditional herbal medicine usage with exhaustive and proper extraction method is an efficient way of identifying biologically active plant materials[11]. Majority of the effective anti-cancer or antibiotic concentrations of plant extracts are greater than 100 g/ml however[12], with extracts of all the plants tested, cell proliferation was inhibited with far less than this concentration.

4. Conclusions

It was found that the cell growth inhibition in the entire cell lines strengthen with increasing concentration of tested compounds. Fractionation revealed that semi polar compound may play a vital role, as chloroform fractions of the plant was endowed with the highest cytotoxic activity against all the cancer cell lines. Compared to reference standard anticancer drug etoposide, the responsiveness of plant extract to cell decreased as etoposide > CHF > HxF > EAF > MeOH = BuF. The responsiveness of cell line to extracts were decreased as SNU-638 >A-549 > A2780 > MIA-PaCa-2. In some cases, the active principles and their derivatives have proven more active than their crude extracts: artemisinin and derivatives[13, 14] (from Chinese *Artemisia annua*) in the treatment of malaria; sitosterols, hypoxoside and rooperol (from African Hypoxis spp.) in the treatment of prostate cancer[15]. Thus our results support that bioactive compounds isolated from the root of *A. pirotta* and their derivatives could prove more active and could exhibit higher anti-A549, A2780, MIA-PaCa-2 and SNU-638 cancer cell lines and inhibiting cancerous cell growth more effectively than standard control compound and many of compounds in clinical use and advanced preclinical trials. Likewise, the broad spectrum extracts and solvent fractions of this plant should be studied for active principles, derivatives, the mechanism of cell growth inhibition, along with further anticancer testing in vitro and in vivo and followed by pre-clinical trials. Therefore, purified compounds as well as their derivatives could hold great promise towards good active leads for the development of a new group of cancer chemotherapeutics agents.

5. Acknowledgements

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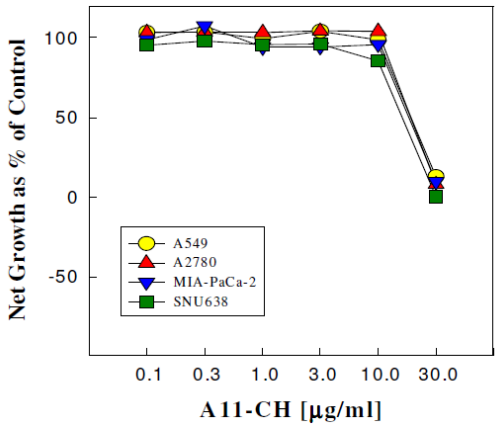
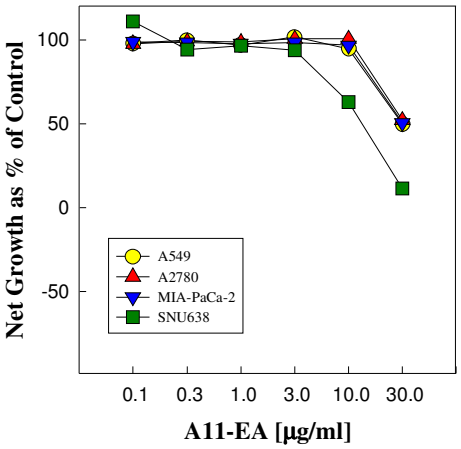
Figure 1: *Aloe pirottae* A. Berger plant



a)



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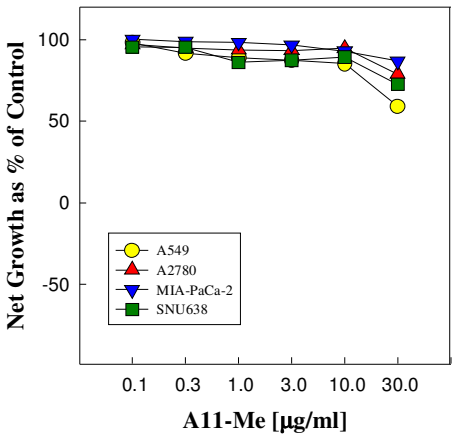

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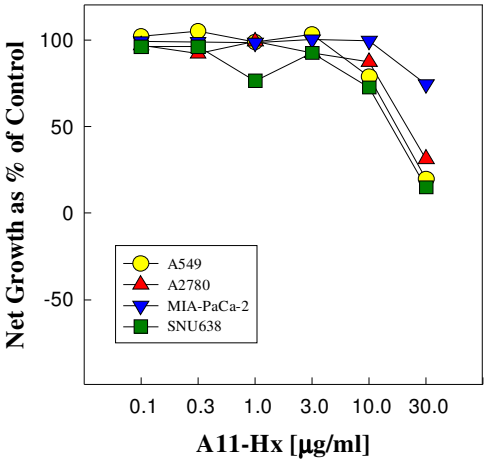

d)

Figure 2: Dose response effects of *Aloe pirottae* A. Berger root extract a. Chloroform (A11-CH), b. Ethylacetate (A11-EA), c. Methanol (A11-Me), d. Hexane (A11-HX), e. Butanol (A11-Bu) extracts on the viability of A549 (human non small cell lung cancer), A2780 (Ovarian cancer), MIA-Paca-2 (Pancreatic cancer), SNU638 (Stomach cancer) cell lines.

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