Euro Surgery 2020: Phytochemical Screening and In-vitro Antioxidant and Antiproliferative Activity of Aqueous Leaf Extract of Ximtenia americana against Non- Small Cell Lung Cancer

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Introduction: Cancer is a general term applied of series of malignant diseases that may affect different parts of body. These diseases are characterized by rapid and uncontrolled formation of abnormal cells, which may mass together to form a tumor or proliferate throughout the body by the process of metastasis. The main forms of cancer treatment for cancer in humans are surgery, radiation and drugs (chemotherapeutic agents) can often provide temporary relief of symptoms, prolongation of life and occasionally cures. Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives every year [1]. In developing countries since from following decades, the numerous of people with cancer will continue to increase may be due to life style, nutrition and environmental conditions [2-4]. In many countries cancer is 2nd leading cause of death after heart diseases [5]. Lung, colorectal and stomach cancer are among the five most common cancers in the world for both men and women [6]. Lung cancer is the leading cause of cancer deaths worldwide. American cancer society estimated in 2016, about 1 of 4 cancer deaths are from lung cancer. Every year, more people die of lung cancer than of colon, breast and prostate cancers. Furthermore if you consider 5 year survival rate for lung cancer patients it drops from 54% to 4% in patients with metastatic lung cancer [7].

However, most of the anticancer drugs currently used such as doxorubicin, paclitaxel give rise to undesirable side effects such as cardio toxicity and tumor drug resistance [8]. Since from ancient time's plant secondary metabolites and their semi synthetic derivatives continue to play an important role in the treatment of cancer as novel drugs [9,10] and 60% of currently used anticancer agents are derived in one way or another from natural sources [11]. Plant derived natural products such as flavonoids, terpenes, alkaloids and phenols are gaining more importance due to their diverse pharmacological properties including cyto-toxic and cancer chemo protective effects [12].

Plants are the rich sources of secondary metabolites such as alkaloids, phenols, flavonoids, tannins, saponins, glycosides, terpenoids etc. that possess a wide array of biological properties including antibacterial, antifungal, antioxidant and anticancer [13]. Phytochemicals and even the whole plant extracts are known to prevent arrest or reverse the cellular and molecular processes of carcinogenesis due to its multiple intervention strategies [14] because of these reason herbal medicines making an impact on both world health and international trade. Medicinal plants continue to play a central role in the health care system of the large proportions of the world's population [15].

However, till-date a systematic study on biological activities of chemical constituents present in X. americana is still not agreeable [23,24]. The extensive literature survey exposed that only few reports exist on this plant leaves, but no information are available on anticancer activity in particular with lung cancer. Henceforth, present study was undertaken and made an attempt to identify Phytochemicals and invitro antioxidant and antiproliferative activity of aqueous extract of Ximenia americana.

Ximenia americana leaves were collected from Karnatak University Campus, Dharwad, India in the month of June, 2017. The leaves were identified and

Note: This work is partly presented at 15th International Conference on Surgery and Anesthesia on August 10-11, 2020 held at London, UK

authenticated by Dr. Kotresha K., Department of Botany, Karnatak Science College, Dharwad, Karnataka, India. A voucher specimen (N0-01/2016) was deposited at the Department of Botany, Karnatak Science College, Dharwad, Karnataka. Fresh disease free plant material was washed under running tap water, shade dried and pulverized to fine powder using mechanical grinder. The powder was stored in airtight containers at room temperature for further use.

Chemicals: 3-(4,5–dimethyl thiazol–2–yl)–5–diphenyltetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA.EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India.

Cell lines: A549 &NCI-H460non small cell lung cancer cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphoteri¬cin B (5 µg/ml) in a humidified atmosphere of 5% CO2 at 37°C until confluent.

Crude extraction: The 100 g of dried X. americana leaf material was extracted with distilled water using Soxhlet apparatus for 4-6 hrs at 40-500°C. The extractant solvent was evaporated using rotary evaporator and the resultant slurry of crude extract was thoroughly dried and weighed. The extract was freeze-dried at −200°C and stored at 40°C until use. The yield was found to be 8% w/w with reference to the air dried plant material.

Phytochemical analysis: The crude powder of Ximenia americana was qualitatively tested for different phytochemical constituents namely alkaloids, flavonoids, glycosides, phenols, lignin's, saponins, sterols, tannins, anthraquinone and reducing sugar by following the standard procedure [25].

Estimation of flavonoids content: The flavonoids content in the plant extracts was estimated according [26] with quercetin as reference standard. It is an aluminium chloride colorimetric method in which each extract (0.5 ml) separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The reaction mixture was kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm using a UV-VIS Spectrophotometer. The value of optical density was used to calculate the flavonoids content present in the sample and the calibration curve was plotted by using quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol.

The antioxidant activity of Ximenia americana was evaluated with ascorbic acid as a standard based on their ability to scavenge the hydrogen peroxide [27]. 0.6 ml of 4mM H2O2 solution in phosphate buffer (pH-7.4) was added to 0.5 ml of known concentration of standard ascorbic acid and to tubes containing different concentrations ranging from 100 µl to 500 µl of plant extracts in phosphate buffer (pH-7.4). Absorbance of the solution was measured at 230 nm after 10 min against the blank solution containing phosphate buffer without hydrogen peroxide. Control was prepared by replacing the sample or standard with phosphate buffer. All samples were assayed in triplicates. The percentage of inhibition was calculated by using formula method.

The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

For Cytotoxicity studies, each weighed test drugs were separately dissolved in DMSO and volume was made up with DMEM supplemented with 2% inacti-

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vated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

The effect of aqueous extract of leaves extract of Ximenia americana on the viability of non-small cell lung cancer (A549 &NCI-H460) cells was determined using the standard colorimetric MTT assay using the 3-(4,5-dimethylthiazol- 2-yl)-2,5-dimethyl tetrazolium bromide dye (Sigma, St. Louis, MO, USA) [28]. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 × 105 cells/ml using DMEM containing 10% FBS and seeded to 96-well microtiter plates (Falcon, Becton– Dickinson, Franklin Lakes, NJ, USA). After 24 h of plating, cells were serum starved for 24 hr. In the present study untreated A549 and NCI-H460 cell lines taken as control group, A549 and NCI-H460 cell lines treated with standard drug Oxaliplatin considered as positive control group whereas aqueous extract of X americana Figure 1 treated A549 and NCI-H460 cell lines taken as treated group. In the present study different concentration of standard drug and aqueous extract of X. americana were taken to study morphological changes as well as cell growth inhibition in both A549 and NCI-H460 non-small cell lung cancer cell lines Figure 2. Morphological studies revealed that compared with control group, treated group and positive control group showed significant increase in detached cells in culture medium. The cells displayed as turgid and shrunken in shape compared to untreated control cells. Morphological changes in nucleus representing apoptosis. Whereas normal cells appeared as regular and normal in shape, in case of treated group chromatin condensation, elongation of cells and decrease in cell count and density were observed which are the characteristic features of apoptosis. Microscopic examination revealed that morphological changes and shrinkage of cells leading to cell apoptosis induced by the aqueous extract of Ximenia americana (Figures 3 and 4).

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