Chemotherapeutic potential of Zanthoxylum armatum fruits and isolated compounds against A459 and HT-29 cell lines

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Abstract

Various advancements have been done in recent years in prevention and cure of cancer. Globally the incidence of colon and lung cancer escalate every year. Plants and their secondary metabolites are tested frequently to explore their effect on this disease. Derivatives of secondary metabolites have been used since long time in order to cure and prevention of disease. Several spices of *Zanthoxylum* are reported for bioactivities including anticancer activity. Fruit of the plant was used as spices traditionally. The *Z. armatum* fruits possess various activities and used by local tribal's in India since vary long time. Traditionally this part of plant is also reported in Ayurvedic Pharmacopoeia along with its important. Numerous activities and chemical identification has been sone in recent years. In the present study *Zanthoxylum armatum* fruits extract including compounds tambulin, ombuin, prudomestin, 2α-methyl-2β-ethylene-3β-isopropyl-cyclohexan-1β, 3α-diol and phenol-O-β-D-arabinopyranosyl-4′-(3″, 7″, 11″, 15″-tetramethyl)-hexadecan-1″-oate were used to test against HT-29 AND A-549 cell lines. Ombuin showed dose dependant scavenging activity against A549 cells. The hexane and butanol extract were also possess good anticancer activity in both mentioned cell lines.

Keywords: *Zanthoxylum armatum* fruits, Anticancer, HT-29, A-549,

Introduction

Disturbance in homeostasis leads cancer, when programmed cell death happens and no self renewal of tissues occurs, from the history drug discovery from natural sources are continue till date. In case of cancer cure, and treatment natural compound like mitomycin D, paclitaxol and taxol leads a milestone [1]. According to Chinese traditional medicine, arsenic is the oldest remedies in china for the treatment of acute promyelocytic leukemia [2]. In US the prevelance of Colorectal carcinoma is elevated compared to other countries worldwide around 52:100000 while in India the ratio is 7:100000. Analyzer predicts that it may be due to lifestyle of individual [3]. Nearly 25% new drug was developed during 1981-2019 for cancer; lot of molecules was isolated from plant, microbes or other living organism. Many natural bioactive compounds known as live saving drug in this era [4], several high rate publications has been reported for occupation associated cancers. According to the report of International Agency for Research on Cancer some viruses, infectious agents, bacteria, fungi etc. may also cause cancer. Various other causes like obesity, sedentary lifestyle, diets and habits may also increases the risk of cancer [5]. Due to poor prognosis the incidence of lung cancer is frequently increasing day by day. Lung cancer is the main leading cause of death in world wide especially in males and about 31% are adenocarcinomas. Silver nitrate has gain significant attention in recent years as it is effective in low dose and having less toxicity [6]. Various available molecules are tested to cure disease like beta-blockers through MTT assay. Propranolol possessed strong activity against H1299. While betaxolol has lower IC50 in A-549 and H1299 cell lines. In a study some neurotransmitter also resist the growth of cancer cell by hindering immune system work by lowering activity and quantity of natural killer cells an lymphocytes [7]. According to report phytomolecule quercitin significantly inhibit the proliferation of HT-29 in dose dependant manner as tested on 50, 100 and 200 µM [8]. *Zanthoxylum* species have been tested for anticancer activity (Drug resistant and drug sensitive) form long time. Stem bark of *Z. clava-herculis* posses antiproliferative lung cancer in A549. Kobusin isolated from *Z. armatum* bark possess pancreatic cell lines (MIA-PaCa) and human lung cancer cell lines (IC_{50} 34.71 and 32.86 µg/ml) [9]. Alkaloids present *Zanthoxylum* possess cytotoxic potential against various cell lines especially in MCF-7. Skimmiaine and fabiocinine reported IC50 value 8.03 and 3.19 µg/ml [10].

Now a day's searching of good analogue carried out by doing structure modification with target delivery are blooming. 277 different types of cancer diseases are known by present era. Identifying stages is itself a key factor in treatment and cure. Genetic disorder or gene mutation are very common problem in existence of cancer. Various new technologies and new methodologies are developed in recent years but the role of natural compounds and searching natural sources are still on full fledge [11]. The present study is aim to investigate the anticancer potential of *Z. armatum* fruit for HT-29, Human Colon cancer cell lines and A-549, Human lung cancer cells.

Material and Method

Plant material

Raw fruit of *Z. armatum* were purchased from Lucknow and authenticated from CSIR-CIMAP. A voucher specimen no ZA/F/1

Extraction and isolation of compound

14.5 kg of crushed fruits were taken and extracted by solvent according to polarity. The extraction was done by cold percolation method. Hexane, ethyl-acetate, butanol and water fraction were collected. Compounds were isolated by doing Column chromatography method with constant monitoring with thin layer chromatography.

Anticancer activity

2 mM L-glutamate was mixed with ten percent fetal bovine serum was used to grow cell lines. Microtiter plates of 96 well were used for inoculation purposes for each cell lines then incubated for 24 hr earlier to introduction of drug at predetermined condition 95% air, 5% CO_2 at 37°C and 100% relative humidity. Samples were dissolved in suitable solvent at 100 mg /ml which was further diluted to 1 mg/ml with water and keep at deep freezer before use. An aliquote of free dried concentrate was liquate and then diluted to various ratios 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. 10 µl of sample dilutions were mixed in micotiter which have 90 µl medium resulting in the required final sample concentrations 10, 20, 40 and 80 µg/ml. Incubate for 48 hr and conclude the assay by incorporating cold TCA (50 µl 30% w/v) and with final concentration of 10% TCA and kept at four degree for sixty min Dispose of the supernatant and plate were washed with the help of water and made air dried. 50 µl Sulforhodamine B solution at 0.4% in 1% acetic acid was dissolved in every well and incubated at room temperature for 20 minutes. After staining recovered the unbound dye remaining dye was eliminated by washing with 1% acetic acid and air dried the plate. 10mM trizma base was added to bound dyes and check the absorbance at 540 nm and 690 nm wavelength. Determine the percent growth and compare with standard control. It is expressed as the ratio of average absorption of test to control wells *100

Six absorbance was recorded for Control growth, test samples at various selected concentrations (Ti) for every sample percent growth was determined

[Ti/C] x 100 %

Result and discussion

Extraction of plant

14.5 kg fruits of the timoor was taken and perform extraction by using methanol by refluxing at every 8 hr for consecutive three days. Collect and concentrate the filtrate, the semisolid mass of 2.9 kg, which is brown in color was obtained and this is further suspended in water and fractionated according to polarity i.e. hexane, ethyl acetate, *n*-butanol successively.

Isolation of compounds

The isolation procedure of mentioned molecules was reported in Nooreen et al., 2017 and 2020 [12-13]. Hexane extract (a), ethyl acetate extract (b), butanol extract (c), aqueous extract (d) along with compounds tambulin (e), ombuin (f), prudomestin (g), 2α-methyl-2β-

ethylene-3β-isopropyl-cyclohexan-1β, 3α-diol (h), phenol-O-β-D-arabinopyranosyl-4′-(3″, 7″, 11″, 15″-tetramethyl)-hexadecan-1″-oate (i)

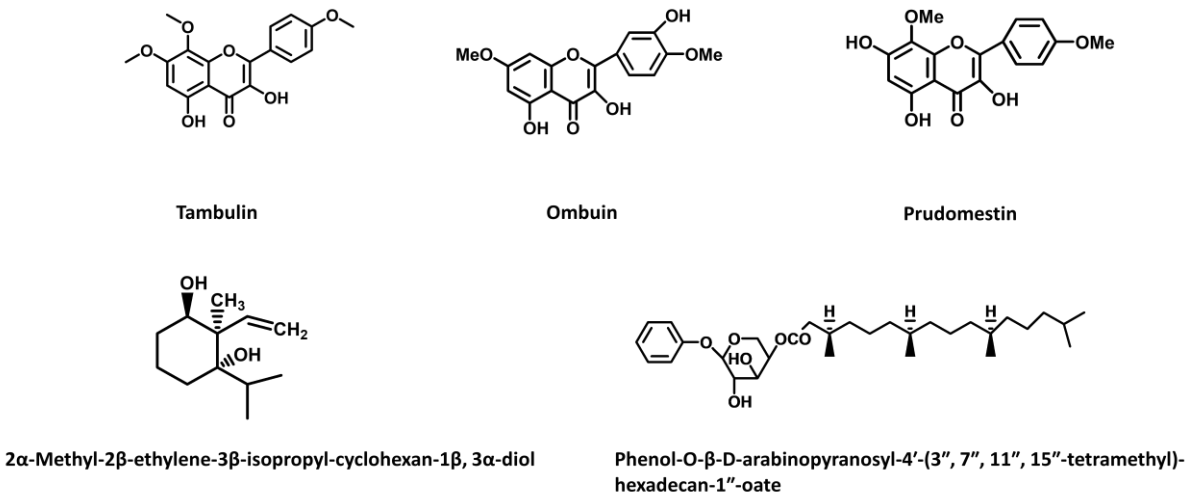
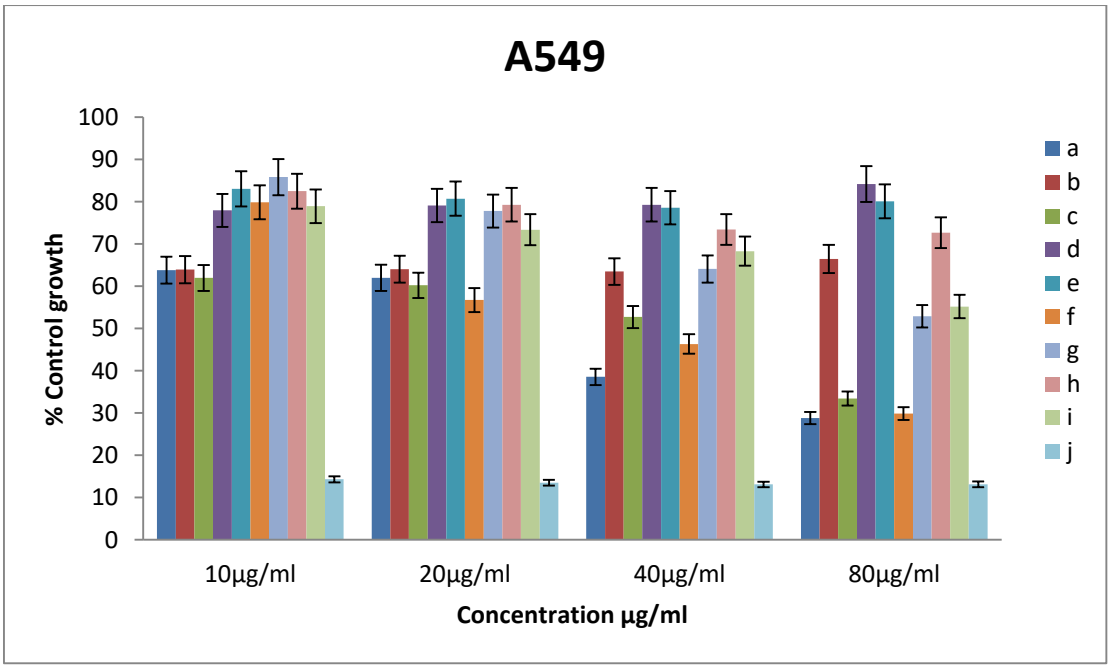


Figure 1. Systematic structure of molecules used to test anticancer activity

Evaluation of apoptosis

Cytotoxicity of dried *Zanthoxylrm armatum* fruit extracts and compounds were determined by using A549 and HT-29 cell lines at different concentrations (10 µg/ml, 20 µg/ml, 40 µg/ml, 80µg/ml) and compared with doxorubicin. Evaluation of result were shown in fig1 and 2. Hexane extract showed good apoptosis against A-549 cell lines at the concentration of 40 µg/ml, 80 µg/ml as 38.55 and 28.81 and butanol as 52.71 and 33.40% and butanol respectively while ombuin possess 29.9% percent control growth at 80 µg/ml concentration and on another hand HT-29 cell apoptosis was observed in dose dependant manner in hexane fraction at the concentration of 40 µg/ml, 80 µg/ml as 66.60 and 53.7% and ombuin possess 56.5% cytotoxicity at 80 µg/ml.



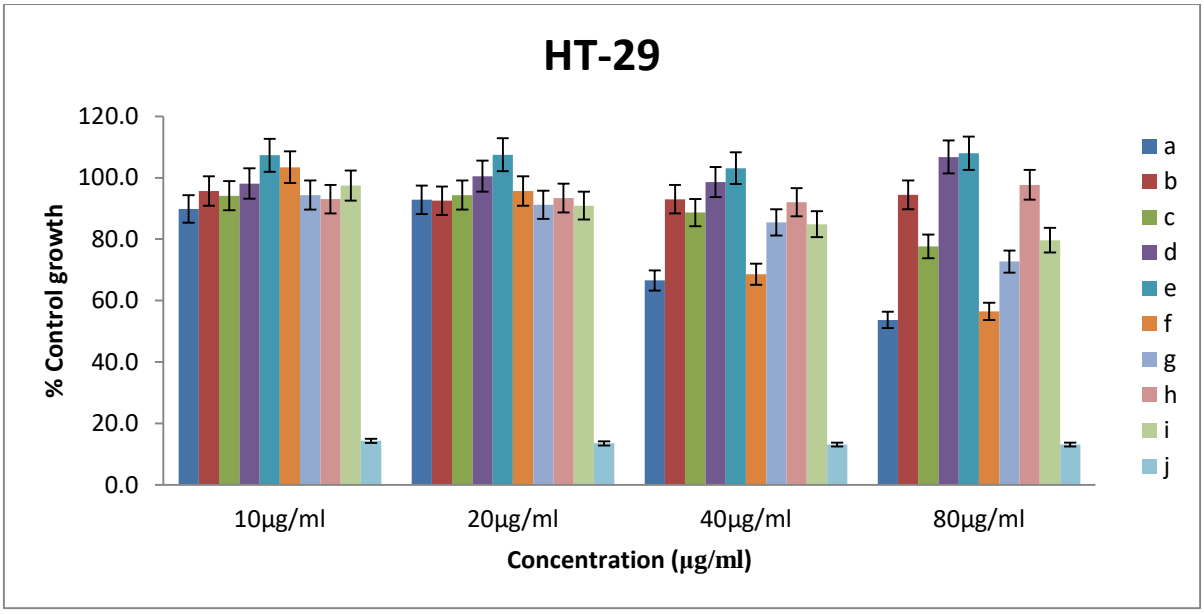


Figure 2. Cytotoxic effect of selected cell lines in hexane extract (a), ethyl acetate extract (b), butanol extract (c), aqueous extract(d), tambulin (e), ombuin (f), prudomestin (g), 2α-methyl-2β-ethylene-3β-isopropyl-cyclohexan-1β, 3α-diol (h), phenol-O-β-D-arabinopyranosyl-4′-(3″, 7″, 11″, 15″-tetramethyl)-hexadecan-1″-oate (i), doxorubicin (j)

Z. armatum i.e Z. alatum was used traditionally from various tribes in India as an spices. They used seed for the treatment of various disorders associated with lungs and stomach. Fruit of the plant was also reported in Indian Pharmacopoeia claims to manage vatta, pitta doshas. Inpresent study we investigate for anticancer potential *Z. armatum* fruit for colon and lung cancer and found that the hexane and ethylacetate extract is useful in the management of A549 (Human lung cancer cells) in dose dependant manner. Ombuin also showed significant activity. Further more the study may be conducted at molecular level to know the exact cause of inhibition in cell growth.

Conclusion

Apoptosis or managed cell death sustain the balance between cell division mechanism, no. of significant cell in various organs. Impairment of these mechanism leads to cellular suicide through membrane blebbing, cell shrinkage and DNA fragmentation. These leads to various diseases and most versatile is cancer. Chemotherapeutic agents are quite effective but carried lots of unacceptible side effect with them. Lots of studies have been reported the use of phytomolecules in the treatment of cancer with low side effects. Introducing of plant based diet in the routine provide a positive effect to the patients[14]. The capability of molecule or fraction to kill a cell independently from programmed cell death is summartized as cytotoxicity. Now this is the most common method to recognize novel mocules and their apoptosis nature. MTT assay was carried out with *A. sessilis* leaves extract against HT-29 cancer cell lines and they posses dose dependant activity [15]. Generally flavonoids are bioactive compounds present almost all the plants. genus *Scutellaria* is traditional spicesn contains various flavonoids in it.

Due to these flavonoidal molecules they are reported form anti-tumor property. *Scutellaria barbat a* leavesethanolic extract possess activity against A540 cell lines having IC_{50} value 0.21 mg/ml [16]. Timoor i.e *Z. armatum* fruit belongs to Rutaceae family commonly found in hill areas, Nepal, India and chaina. It is also called ad thorny ash tomar or toothache tree by common people. It has been used as flavor and aroma in many dishes especially which are spicy. Phytocemically the plant possess flavonoid, glycosides, lignans and phenolic compounds etc. the plant has been associated to possess antioxidant, antimicrobial, larvicidal, antipyretic and hepatoprotective action. From some recent years the production and demand of the fruit of plant increases to million tones. Many researchers also investigate for anticancer properties but this part is not done so far. Cytotoxic activity of *Z. alatum* was tested followed by antioxidant properties. Methanolic extract showed significant antioxidant property thus selected for anticancer activity. The extract showed significant cytotoxicity against Ascites cancer cells having IC_{50} 102.30±0.81µg/mL [17]. In study *Z. armatum* leaves, bark, fruit methanolic extract were tested on Caco-2 , MDA-MB-468 and MCF-7 cell lines by MTT assay and found apoptosis in Caco-7 cell as 53.16%, 66.43% and 45.96% respectively when compared to Actinomycin-D While others were not having significant change in cells [18].

Many research activities have been done to explore *Z. armatum* fruit was done in recent years but this expect was untouched by the researcher. Current research was aimed to investigate the potential of *Z. armatum* fruit in two cell lines A549 and HT-29. Our finding suggest that hexane and butanol fraction may further evaluated at molecular level as both of them possess good anticancer activity in A549 cell lines.

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Conflict interest

No potential conflict of interest was reported by the authors.

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Ethics statement: None

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