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Evaluation of *in vitro* anticancer potential in *Punica granatum, Psidium guajava, and Vitis vinifera* seed extracts

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ABSTRACT

The anticancer potentials of the seed extracts of edible fruits pomegranate (*Punica granatum* L.), guava *(Psidium guajava* L.) and grapes (*Vitis vinifera* L.) were evaluated. Doxorubicin was used as a reference drug. MTT assay was used to determine the anticancer potential of the selected seed extracts. Among the selected edible fruit seeds and their different organic solvent extracts, the ethyl acetate extract of *Punica granatum* possessed a higher inhibitory effect against lung cancer cell line, compared to other solvent extracts of *Psidium guajava* and *Vitis vinifera* fruit seeds, and the IC_{50} value was 51.25 ± 1.25 µg/ml, 60.21 ± 1.35 µg/ml and 61.21±1.45µg/ml, respectively. The IC_{50} of doxorubicin was 49.25 ± 1.85 µg/ml. The inhibitory effects of fruit seeds against lung cancer cell line (A549) could be ranked as *P. granatum* > *P. guajava*. >*V. Vinifera.* In the overall observations of the study, the *P. granatum* seed extract showed the highest inhibitory effect on lung cancer cell line among the other seeds.

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INTRODUCTION

Cancer is a major public health problem worldwide and is the second leading cause of death in the United States. Each year, the American Cancer Society estimates the number of new cancer cases and deaths that will occur in the United States and has compiled the most recent data on cancer incidence, mortality, and survival (Rebecca *et al*., 2018). Reports of newer cancer cases and cancer death rates will increase in future in the USA. The prevalence of lung cancer was higher than any other form of cancer. In India, lung cancer constitutes 6.9% of all new cancer cases and 9.3 of all cancer-related deaths in both men and women (2018, WHO).

According to the latest reports, cancer will be over 13.1 million in 2030 worldwide. (Wisastra *et al*., 2014). Expensive facilities and drugs are required in the treatment of cancer. It is a known fact that growing cancer cells may harm healthy cells and cause side effects too such as autoimmune reactions, damage to the heart, thyroid gland, liver and kidney, slow wound healing, increased risks of infections and blood clotting problems throughout the body. According to the latest discoveries, finding efficient, non-toxic anticancer agents with less side effects has become imperative to give effective therapeutic regime that provides safe and reliable treatment (Ashraf *et al*., 2015). The therapeutic humanity arsenal, have been considered from the plants (Santos *et al*., 2017). Over the years they have come as a major source of bioactive molecules, providing more phytoconstituents to the pharmaceutical industry. Secondary metabolites

possessing many pharmacological properties, especially antioxidant molecules, have got much attention. Recently a good number of prescriptions have got one or more active ingredients from plants. Hence plant sources have led to the discovery of anticancer drugs. (Durdevic *et al*., 2018).

WHO recommends herbal medicines to cure various illnesses and has reported that nearly 80% of the people worldwide are using medicinal plants for various treatments (Cock *et al*., 2018). In the scientific community, various medicinal plants and their active phytoconstituents are reported to be effective against lung cancer: some of them are Broccoli, Witch-hazel, grape fruit, Brussels sprouts, apples, their seeds and fruits containing kaempferol which induce changes in the nuclear factor kappa B (NF-κB) pathway (Cui *et al*., 2008), wheat germ oil and sunflower oil seed containing alpha-tocopherol compound which inhibit the cytotoxic effects of cigarette smoke (El-Hallouty *et al.,* 2015). The seeds of red capsicum are anti-angiogenic to suppress tumour growth, anti-metastic and anti-mutagenic (Venier *et al*., 2015). Peanut, grape, mulberry, bilberry and blueberry seeds and roots contains resveratrol for modulation of (AMPK) adenosine monophosphate-activated protein kinase signalling pathway for the starvation of cancer cells (Wisastra *et al*., 2014). Grape seed pomace has anthocyanins which suppress BC cyclooxygenase activity of the enzyme and inhibits the growth of cell (Pihlava *et al.,* 2018), Tomato fruit contains high lycopene content, which has anti-invasive functions through Akt and ERKs signalling pathways (Petchsak *et al*., 2015). *Garcinia indica* fruit and seeds produce garcinol that plays a role in inhibiting cell growth like MCF-7 and MDA-MB-231 (Ahmed *et al*., 2015), *Camellia sinensis* flowers and seeds are rich in catechins that assist in up-regulation and expression of the anti-oxidases like superoxide dismutase (Sekine *et al.*, 2018). Quercetin from lemon, tomato, apples, red wine, tea, onions and broccoli fruit possesses the ability to inhibit cancer through miRNA expression (Bakshi *et al*., 2016).

The present study reveals the anticancer properties of the seed extracts of edible fruits, the seeds being a waste from the fruit juice industry. The seeds of *Punica granatum*, *Psidium guajava* and *Vitis vinifera* have been collected and analyzed to study their curative properties against lung cancer.

MATERIALS AND METHODS

Fruit material collection and extraction

The seeds of *Punica granatum*, *Psidium guajava* and *Vitis vinifera,* rendered waste in the juice industry at Coimbatore, Tamil Nadu were collected for analysis.

The powder of *Punica granatum*, *Psidium guajava* and *Vitis vinifera* seeds (100g) was extracted exhaustively by the use of organic solvents (petroleum ether, ethyl acetate, chloroform, methanol and ethanol) in the ratio of 1:5 (w/v) for 12 hours by using a Soxhlet apparatus with 5-6 successions. The extracts were dried using rotary flash evaporator to get solid form.

Cytotoxicity studies

Cell culture

The lung cancer cell line (A549) was grown in Eagles Minimum Essential Medium containing 10% fetal bovine (FBS) after it was obtained from National Centre for Cell Science (NCCS). The cells were maintained at 37˚C, 5% CO_2, 95% air and 100% relative humidity; the culture medium was changed twice in a week, and Maintenance cultures were passaged weekly.

Cytotoxocity by MTT assay

Cytotoxicity potential of the selected fruit seed extracts against lung cancer cell line (A549) was assessed by MTT assay (Mosman *et al*., 1983). The cells cultured in flat-bottomed, 96-well tissue culture plates and treated with ethyl acetate and methanol extracts of *Punica granatum*, *Psidium guajava* and *Vitis vinifera* seed extract were chosen based on the presence of more secondary metabolites at the concentrations of (6, 12, 25, 55, and 85 µg/ml, and doxorubicin was used as a standard drug. According to the experimental design the cells were treated, and incubation time was optimized for every cell type and system. The tetrazolium compound MTT (5mg/ml) was added to the wells, and the cells were incubated. MTT was reduced by metabolically active cells to insoluble purple formazan dye crystals. The detergent was then added to the wells, solubilizing the crystals and the absorbance was read at 570 nm using a spectrophotometer. The data were analyzed by plotting concentration of extracts versus absorbance, allowing quantitation of changes in cell proliferation and % inhibition. The formula below was used to determine the % cell inhibition.

% cell Inhibition = 100- absorbance (sample) /absorbance (control) x100.

The graph of nonlinear regression was plotted between the % inhibition of the Cell to find the 50% inhibitory concentration of extracts concentration (Chen *et al*., 2015; Ravindranathan *et al*., 2018).

RESULTS AND DISCUSSION

The IC_{50} value of the methanolic extract *P. granatum, P. guajava* and *V. vinifera* was 52.11 ± 1.15, 61.01 ± 1.15 and 63.21 ± 1.45 µg/ml, respectively. While that of ethyl acetate extract of the edible

fruit seeds such as *P.granatum, P.guajava* and *Vitis vinifera* were found the IC_{50} value as 51.25 ± 1.25 µg/ml 60.21 ± 1.35 µg/ml and, 61.21 ± 1.45µg/ml respectively, the IC_{50} value of doxorubicin was 49.25± 1.85 µg/ml (Figure 1-3). The lower IC_{50} indicates higher anticancer properties. Cell inhibition was higher in the ethyl acetate extract of *Punica granatum* fruit seed, compared to the seed extracts of other edible fruit, *Psidium guajava* and *Vitis vinifera*. Venugopal *et al.,* 2017 reported that the Inhibition concentration (IC_{50}) from phytomediated AgNPs were read at 50 µg/ml^{-1} A549 and 60 µg/ml^{-1} against MCF7 cells. The viability of the cell was usually evaluated by MTT and for the screening of the drug cytotoxicity. The reduction of MTT (yellow coloured) with other dyes of tetrazolium depended upon the activities of cellular metabolic due to NAD (P) H-dependent cellular oxidoreductase enzymes (Shaikh *et al*., 2014).

Anticancer effects of selected fruit seed extract on A549 cell line.

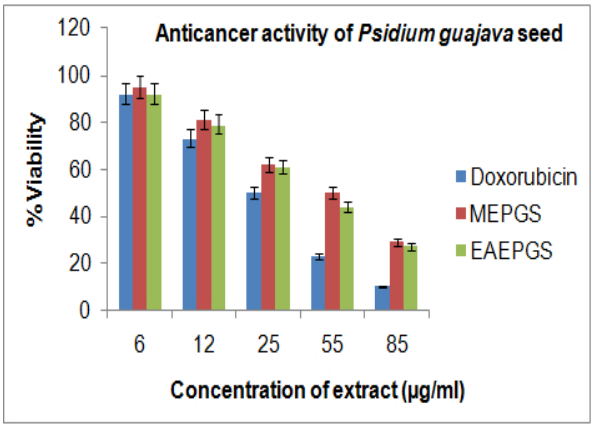

Figure 1: Cytotoxicity effect of *Psidium guajava* seed extracts on lung cancer cell line
(MEPGS-Methanol extract of *Psidium guajava* seed; EAEPGS-Ethyl acetate extract *of Psidium guajava* seed)

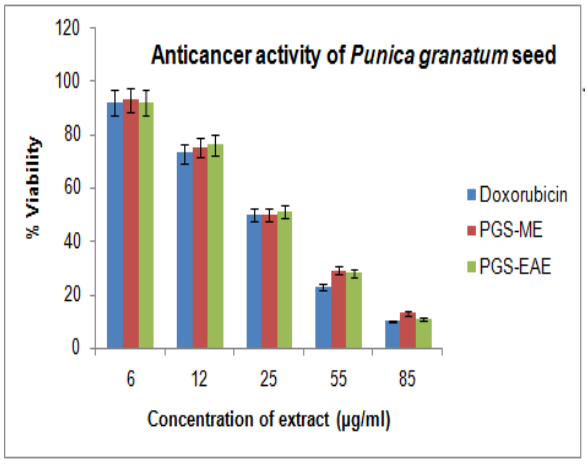

Figure 2: Cytotoxicity effect of *Psidium guajava* seed extracts on lung cancer cell line
(PGS-ME-*Punica granatum* seed methanol extract; PGS-EAE-*Punica granatum* seed ethyl acetate extract)

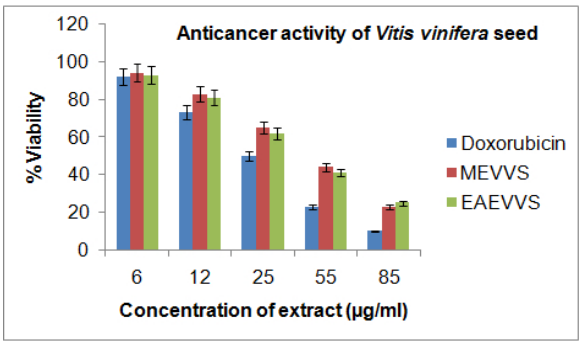

Figure 3: Cytotoxicity effect of Vitis vinifera seed extracts on lung cancer cell
(MEVVS-Methanol extract of *Vitis vinifera* seed; EAEVVS-Ethyl acetate extract of *Vitis vinifera* seed)

Cytotoxicity effects of selected fruit seed extract on A549 cell line.

The growing healthy cells show high rates of MTT reduction in formazan, while dead cells fail. The purple color formation, from the final product of MTT reduction, can be easily dissolved in DMSO. Viability in the MTT assay is connected with the quantification of formazan at 570 nm, which is linearly associated with the enzyme activity and indirectly the number of viable cells. The visibility of more purple color indicates the viability of cells and the minimal color shows the decreased number of the cells, hence cytotoxicity of a given extract (Figure 4). (Shaikh *et al*., 2014; Twilley *et al*., 2017). The novel bioactive compounds with multitargeting efficacy are needed, due to the complexity of cancers, and here we evaluated the anticancer potential of the seed extracts of edible fruit.

In cytotoxicity studies, the selected fruit seed extracts were screened for their anticancer properties on lung cancer cell line, and the results showed that the ethyl acetate extract of *Punica granatum* seed extracts contained higher inhibitory activity than those of *Psidium guajava* and *Vitis vinifera.* The viability percentages of A549 cells was reduced by 80% in the presence of the extract compared to *Psidium guajava* and *Vitis vinifera* seeds (Eskandani *et al*., 2014). The cell viability decreased with the increasing concentrations of the seed extracts of edible fruit, and the logic might be due to the stimulation of reactive oxygen species (ROS) by edible fruit seed extracts and by their action on cellular constituents that led to apoptosis (Trigne *et al*., 2013). The results obtained in MTT assay showed that ethyl acetate extract of *Punica granatum* seed was the most active amongst *Psidium guajava* and *Vitis vinifera* seed extracts.

Finally, the results of this study highlighted that, the ethyl acetate extract of *Punica granatum* fruit

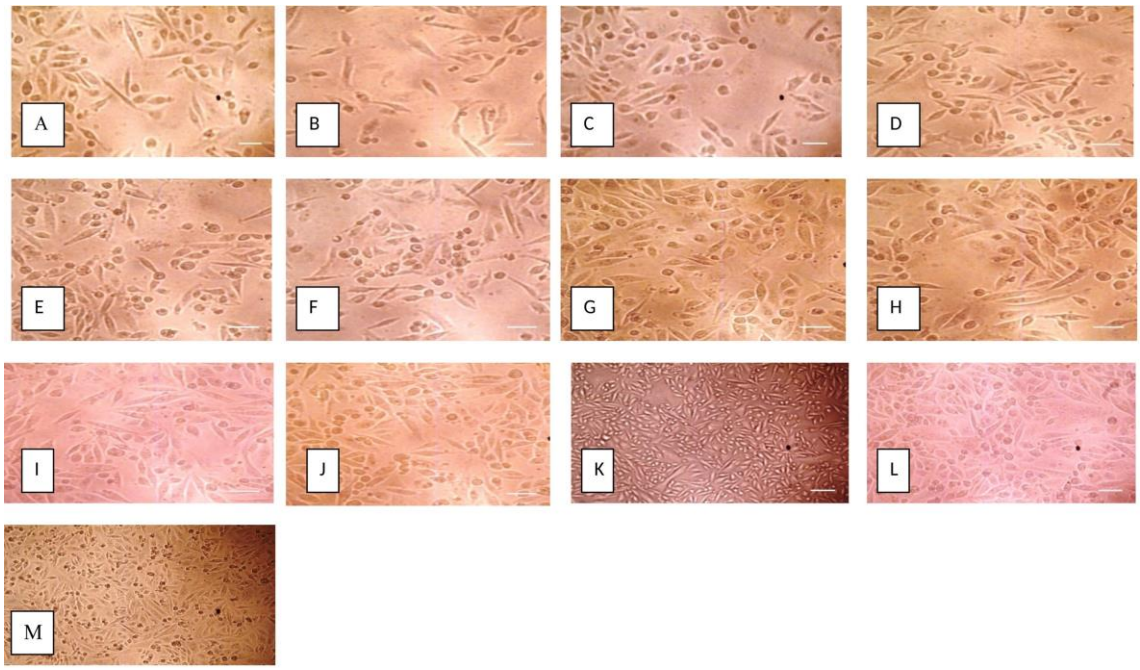

Figure 4: Effects of edible fruit seed extract along with standard drug doxorubicin on A549 cell line under controlled conditions, 200x magnification

(A-EAEVVS IC_{50}-Ethyl acetate extract Vitis vinifera seed; B-EAEPGS IC_{50}-Ethyl acetate extract Psidium guajava seed; C-PGS-EAE IC_{50}-Punica granatum seed ethyl acetate extract; D-MEVVS IC_{50}-Methanol extract of Vitis vinifera seed; E-MEPGS IC_{50}-Methanol extract of Psidium guajava seed; F-PGS-ME IC_{50}-Punica granatum seed methanol extract; G-EAEVVS-Ethyl acetate extract of Vitis vinifera seed; H- EAEPGS-Ethyl acetate extract of Psidium guajava seed;I- PGS-EAE-Punica granatum seed ethyl acetate extract; J- MEVVS-Methanol extract of Vitis vinifera seed; K- MEPGS-Methanol extract of Psidium guajava seed;L- PGS-ME-Punica granatum seed methanol extract; M-Control)

seed. Therefore, this led to further isolation of active constituents from ethyl acetate of *Punica granatum* seed extract (Santos *et al*., 2017; Bishayee *et al*., 2016).

CONCLUSION

In conclusion, the cytotoxic potential of the seed extracts of edible fruit was highlighted in this study, especially ethyl acetate extract of *Punica granatum,* which was able to inhibit the growth of lung cancer cell line (A549). This would help find out further active principal isolation and characterization of molecules from the seed extract of *Punica granatum*. Generally, the use of synthetic drugs produces more side effects. But, through the use of proven phytomolecules of fruits, it has been reported earlier too, new phyto drugs with minimal side effects would be possible.

Conflict of interest statement

The authors declare that they have no competing interests.

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