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Cell Death Induction Potential in Seed Extracts- Hidden and Bioactive Phytochemical Treasures

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ABSTRACT

Seeds have been known to possess bioactive components with anti-cancer properties. This study aims to demonstrate the processes by which seed extracts from various sources induce cell death. Several assays have been employed to demonstrate the induction of cell death by the respective seed extracts. This review also underscores the importance of Grape Seed Proanthocyanidins (GSPs) in terms of inducing the aforesaid physiological form of seed extract-induced cell death. Furthermore, this review highlights the critical and pressing need to conduct comparative HTS-based strategies (with a battery of cell lines representing different cancers) to identify the major seed extracts that can reproducibly serve to augment the cell death induction capabilities of the existing battery of chemotherapeutic drugs/ natural alternatives.

INTRODUCTION

Seed extracts are known to be rich in phenolic and flavonoid compounds, minerals, vitamins, fatty acids. Such extracts and bioactive compounds extracted from various seed sources have exhibited therapeutic potential in drug development for various human diseases and disorders. They have exhibited high antioxidant and anticancer properties (Liu et al. 2016), anti-inflammatory (Chene et al. 2016), antibacterial (Al-Mamun et al. 2016), and hepatoprotective effects (Mahli et al. 2015, Ogaly et al. 2015). In terms of its anti-cancer properties, demonstration of the improved induction of cell death would be the proof-of-concept strategy for screening and selecting the best seed/solvent(s) combination for anticancer drug development. This review will address a gap in the literature by critically analyzing existing research, discussing future challenges, and bringing readers up to date on seed extract-mediated cell death (*in vitro* and *in vivo*). The rationale behind this approach is that cell death resistance, exhibited by tumorigenic and their relatively recalcitrant subsets (stem cells) is one of the hallmarks of cancer. Hence, the administration of extracts from seed sources may be a feasible and viable strategy for cell death induction. Our approach of selecting and discussing research papers based on work carried out on extracts of seeds from diverse sources would provide an impetus to perform High-Throughput Screening (HTS) of the various extracts and/or the bioactive components contained therein. This strategy would enable us to rank order them in terms of their efficacy in inducing cell death. Also, the experimental design should ensure that the various seasonal and dormancy-related variations in the levels of the bioactive components are taken into account. Last but not least, this experimental design can be extended to evaluate crude extract-synthesized natural molecule derivative combinations for possible synergy in cell death induction potential.

Selected Seed Extracts and Apoptosis -*In vitro* Approaches

A plant extract-based chemotherapeutic strategy can complement surgery and radiotherapy and possibly reduce, if not eliminate, the undesirable, inevitable side-effects associated with the current arsenal of anti-cancer drugs. A vast majority of FDA-approved new plant compounds are used for cancer therapy and have shown good results in clinical trials and therapeutic applications (Seca & Pinto 2018). The papers cited and discussed concern mechanistic information about the involvement of either or both extrinsic and intrinsic mechanisms of cell death, as well as empirical measurements of seed extract-induced cell death. In certain cases, *in vitro*, antioxidant assays have been employed to correlate ROS levels with cell death induction. This paper gives an overview of the cell death-inducing potential of extracts from a variety of plant flora that is indigenous to different parts of the world. This approach highlights the ubiquitous presence of

significant bioactive molecules in seeds, despite qualitative and quantitative differences in their content, warranting a systematic screening of seed extracts for the selection of the best seed/extract-cancer pair for cell death induction.

Acacia belongs to the Fabaceae family and Mimosoideae subfamily, seen predominantly in the African and Australian continents. Cytotoxic effect of ACS extract was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, using concentrations of 0.1–1000 $\mu g.mL^{-1}$ for 24 h. A. catechu ethanol seed extract was treated SCC-25 cells with 25 and 50 $\mu g.mL^{-1}$. At the end of treatment period, apoptotic marker gene expressions such as caspase 8, 9, Bcl-2, Bax, and cytochrome c were evaluated by semiquantitative reverse transcription-polymerase chain reaction. Morphological changes of ACS treated SCC-25 cells was evaluated by acridine orange/ethidium bromide (AO/EB) dual staining. Nuclear morphology and DNA fragmentation was evaluated by propidium iodide (PI) staining. *Areca catechu* ethanol seed extract treatment caused cytotoxicity in SCC-25 cells with an IC50 value of 100 $\mu g.mL^{-1}$. Apoptotic markers caspases 8 and 9, cytochrome c, Bax gene expressions were significantly increased upon ACS extract treatment indicate the apoptosis induction in SCC-25 cells. This treatment also caused significant downregulation of Bcl-2 gene expression. Staining with AO/EB and PI shows membrane blebbing, and nuclear membrane distortion further confirms the apoptosis induction by ACS treatment in SCC-25 cells (Lakshmi et al. 2017).

Adenium obesum is a flowering plant, which is part to the Apocynaceae family and is indigenous to certain parts of Africa. The cytogenotoxic potential of *Adenium obesum* seed extracts was evaluated in MCF-7 cells. A growth inhibition assay was performed and it was shown that the IC50 of a crude Methanol-based extract was 337 $\mu g.mL^{-1}$. After a 12hour exposure to 200 $\mu g.mL^{-1}$ and 300 $\mu g.mL^{-1}$ of a methanolic extract of this seed, flow cytometric evaluation showed that 37% and 35% of cells were in the early and late apoptosis stages. At the same time point and identical concentrations, the results of the comet assay provided adequate evidence in terms of the total DNA damage score being 614 and 617 respectively. Due to the aforesaid demonstrated properties, this extract may be exploited and further evaluated for its anticancer potential (Ali et al. 2019).

The avocado, a flowering plant, has been reported to originate from certain parts of Mexico and is a member of the Lauraceae family. Free radical scavenging capabilities of the Colored Avocado Seed Extract (CASE) was determined using the Oxygen Radical Acceptance Capacity (ORAC) and the lipid hydroperoxides reduction assays. At a concentration of 500 $\mu g.mL^{-1}$, the ORAC value was 2012 ± 300 Trolox equivalents/mg. Further, there was a 33% reduction in the lipid hydroperoxide formation (emulsion was oil-in-water), at the same concentration of CASE. In terms of the half-maximal effects (19 to 132 $\mu g.mL^{-1}$), a wide range of inhibitory concentrations was measured, following a 48hour *in vitro* exposure of a battery of cell lines (MCF7; H1299; HT29; LNCaP) to the CASE. In LnCaP cells, the IC50 value for CASE was 42 $\mu g.mL^{-1}$ at the 12hour time point. A G_0/G_1 cell cycle arrest was observed and the expression of cyclin D1 and E2 (Western blot (WB) assay) were decreased, despite differences in the exposure period. The extent of the cell cycle arrest was 70 and 84% respectively at the IC50 and IC60 concentrations. Concentration-dependent elevations in cell death were determined based on Annexin V-FITC/PI measurements of phosphatidylserine externalization. In the same cell line, WB assay showed that cell death was correlated with elevations in caspase-3 expression as well as cleaved PARP levels relative to control values. Similarly, WB results showed a correlated decrease in the translocation of NF-kB to the nucleus (Davatgaran et al. 2017).

The Apricot belongs to the *Prunus* genus and its species is Armeniaca, even though related species are also considered to form part of this family. Variations in cellular behavior were demonstrated depending on the source of the kernel extracts at the 100, 500, and 1000 $\mu g.mL^{-1}$ concentrations respectively. The main bioactive principle in the kernels associated with cyanide production is amygdalin. This bioactive component inhibits cytochrome c oxidase –an important enzyme in the Electron Transport Chain (ETC). This, in turn, affects ATP production, and hence, this results in the shift from apoptosis to necrosis. The extracts from the Chinese apricots (CAK) produced a time and concentration-dependent decrease in cell numbers following incubation for 24 as well as for 48 hours. However, after exposure for 3 days, solvent effects were seen in the case of the 100 $\mu g.mL^{-1}$ (CAK-hydrophilic) as well as in 1000 $\mu g.mL^{-1}$ (CAK-hydrophilic and CAK-lipophilic). However, biphasic temporal and concentration-dependent variations were demonstrated in HT-29 cells by all the apricot kernels of the South African variety. At the 24-hour time point, the South African apricot (SAK) kernels increased cell proliferation at the 100 and 1000 $\mu g.mL^{-1}$ concentrations, while the cell numbers decreased at the intermediate 500 $\mu g.mL^{-1}$ concentration. Similar, albeit statistically insignificant, effects were observed following a 48hour incubation period. Marked variation in this cellular behavior was exhibited at 72 h. As in the case of the 24hour time point, the lowest, as well as the highest concentration of all the SAK extracts, tested inhibited cell proliferation. However, there was a substantial increase in cell numbers, following exposure to 500 $\mu g.mL^{-1}$ of the extract. A similar trend was seen in the case of the extracts from the South Af-

rican Peach kernels (SPK). The lack of a correlation between the morphological changes and possible apoptotic-related phenomenon (Hoechst33342-based staining) indicated that their observed irregularities in shape as well as in cell volume were plausibly not linked to apoptosis in the case of the SAK and SPK extracts. In the case of the 100 $\mu g.mL^{-1}$ Chinese peach Hydrophilic (CPK-H) extract (24 h exposure), membranous protrusions (blebbing) was seen, while at 48 hours, the Chinese Apricot Total (CAK-T) extract at a concentration of 100 $\mu g.mL^{-1}$, exhibited irregularities in the cellular morphology. In general, the cell cycle analysis data (of all the extracts) was that there was a significant increase in the S phase cells, despite the lack of a definite dose-dependent trend. The temporary nature of the block may be attributable to the cell being able to withstand the damage during the process of replication. This may explain the observed decrease in cell numbers following a 24-hour exposure to the Chinese kernel extracts (Cassiem et al. 2019).

Alcea rosea is an ornamental plant in the Malvaceae family. The ethyl acetate extract of *Alcea rosea* (AR; Hollyhock) was tested (hexosaminidase assay) at a concentration range of 0-100 $\mu g.mL^{-1}$) for its anti-proliferative effects in two colon cancer cell lines (HCT116 and SW480) at three different time point (24, 48 and 72 hours). Results clearly indicated the dose and time-dependent nature of the growth inhibition in both the cell lines with a correlated decrease in Ki-67 (a marker indicative of the proliferation status). In both the cell lines, these extracts inhibited the cells in the transition between the G_0 and G_1 phase at 24 and 48 hours. In addition, this block could be correlated with a decrease in the levels of Cyclin B and D1 based on WB data. The apoptosis data, as well as the molecular correlates (cleaved PARP; decreases in Bcl-xL; an elevation in the Bax levels and an increase in fully cleaved caspase-3), provided fairly definitive evidence of this AR seed extract's capabilities to induce cell death by apoptosis. Next, the colony-forming potential was assessed following treatment of both cell lines with AR for a week. Serial passaging and replating were done to demonstrate the dose-dependent decrease in the number and size of colon spheres (thereby providing inferential evidence of a decrease in the number of stem cells). WB analysis of cell lysates obtained from 2D cultures of both cell lines was done, after their treatment with the AR extract. In this 2D model system, a decrease in the stem cell markers was observed (ALDH1A1, Dclk1, and CD44e), and this may be correlated with the decrease in the number and size of colon spheres. This data was correlated with a concomitant decrease in β-catenin, plausibly linked to increases in WIF1 (a Wnt antagonist). The increased expression of the antagonist could be correlated with decreases in EZH2 signaling (an epigenetic regulator involved in the expression of the antagonist). Also, components of the Notch pathway (Notch-ICD and Hes1) were decreased following treatment with AR extract. Due to its apoptotic potential, analytical characterization would aid in the identification of the probable bioactive component, that may have most plausibly contributed to this controlled form of cell demise. In this regard, HPLC-based preliminary analysis has provided them with some pointers for the delineation of the principal active components that can be developed to target colon cancer (Ahmed et al. 2016).

Cell line studies have shown that *Aesculus hippocastanum* (the horse chestnut –flowering plant belonging to the soapberry and lychee family Sapindaceae) seed extracts have several pharmacological properties, including anti-inflammatory and cell death induction potential. The major bioactive principle is considered to be β-escin or escin (Penta tricyclic terpene). However, this report is the first of its kind, wherein the cytotoxicity ad cell death potential of escin was evaluated using C6 glioma and A549 cells. The combination of cytotoxicity and cell death assays employed include MTT, Annexin V–FITC. This design was extended to measure Caspase-3 and Bax protein expression, apart from TEM-based detection and correlation of the escin-induced apoptosis with the other cell death assays. Based on the MTT assay, the IC50 values for the C6 glioma and A549 cells respectively were 23 and 16.3 $\mu g.mL^{-1}$ (24 and 48-hour exposure) and 14 and 11.3 $\mu g.mL^{-1}$ (24 and 48-hour exposure). In the flow cytometry assay to detect apoptotic cells, at the concentration range tested (3.5, 7, 14, 21 $\mu g.mL^{-1}$), the early and late apoptotic events were 1.6, 6.0, 26.2, 31.6% and 2.4, 4.6, 7.1, 32.2% respectively. Also, it was reported that the relative sensitivity of A549 cells was greater than the C6 glioma cells in terms of escine-induced apoptosis. This finding was corroborated by TEM-based imaging (14 and 21 $\mu g.mL^{-1}$) wherein cell rounding; alterations in the cellular organization; cell shrinkage, DNA condensation, and disruption of the organelle membranes were observed. At the 7 $\mu g.mL^{-1}$ concentration, the vacuoles containing cellular material were evident along with slight damage to the membranes. Caspase-3 activity was measured using flow cytometry following a 24 exposure of A549 cells to escin-the major bioactive principle. Results clearly indicated at doses (3.5, 7, 14, and 21 $\mu g.mL^{-1}$), there was a dose-dependent increase in the caspase-3 positive cells (2.6, 8.4, 10.3, and 13 % respectively). Immunohistochemical findings documenting the induction of Bax at the 14 and 21mL concentrations provided indirect evidence of the involvement of the mitochondrial pathway in escin-mediated cell death in A549 cells. In terms of the cell cycle analysis, a general trend was observed in terms of an increase in the concentration resulting in a greater number of cells arrested at the G0/G1 phase (even though there is some discrepancy

between the information in the body of the text and the tabular column) (Ciftci et al. 2015).

The citrus fruits, belonging to the citrus family, are very popular throughout the world and are horticulture crops. Bioactive molecules were extracted from the various parts of the citrus fruit. Specifically, this study involved the identification of hesperidin, neohesperidin, and naringin. Subsequently, MTT-based cytotoxicity measurements were made following the treatment of HepG2 cells to either the crude ethanolic extract or bioflavonoids (0-200 $\mu g.mL^{-1}$) for 24 hours. The IC50 value is approximately 200 $\mu g.mL^{-1}$ with the bioflavonoids causing a greater decrease in the cell viability. In terms of toxicity of hesperidin, naringin, and neohesperidin, the IC50 values showed that Hesperidin was the most toxic to HepG2 cells with an IC_{50} value of 150.43 ± 12.32 μM. At all the concentrations tested, the IC10 (15%), IC20 (19.7%) and IC50 (22.3%) values for Hesperidin provided evidence of a dose-dependent increase in early apoptotic cells. This result was based on flow cytometric findings, following staining with Annexin V-FITC/ PI. The role of certain initiator caspases (Caspase-9 and Caspase-8), as well as the principal executioner caspase (caspase-3), was evaluated based on their ability to cleave p-NA (a substrate which can be detected based on its λmax at 410 nm) from substrates that are selective for each of the three caspases. The leakage of a dye into the cytoplasm indicative of the loss of the mitochondrial transmembrane potential was observed in Hesperidin-treated HepG2 cells. Dose-dependent increases in the leakage of this dye (ionic fluorochrome, 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6$)) and the consequent decrease in fluorescence intensity was observed in these cells. An interesting finding was the absence of induction of ROS levels, based on the fluorescence intensity changes in Hesperidin-treated HepG2 cells at the 4hour time point (DCFH-DA assay). Further, the earlier demonstration of dose-dependent increases in apoptosis was further corroborated based on the down-regulation of the anti-apoptotic Bcl-xL protein, whereas there was an increment in the Bax, Bak, and tBid protein levels the involvement of t-bid provides evidence of cross-talk between the intrinsic and the extrinsic pathways in hesperidin-mediated cell death in HepG2 cells (Banjerdpongchai et al. 2016).

Dorema glabrum is a member of the Apiaceae family and is widely prevalent in North-Western; South Western and Central Asia. Limited data on the root-based bioactive principles of this plant is available. However, this study reports on the anti-cancer potential of seed extracts of this plant. Extraction; fractionation followed by preparative HPLC resulted in the isolation of three phenolic compounds, including diglucosyl caffeoyl ester; 4-O-β-D-glucopyranosylcaffeic acid, and Umbelliferone 7-O-β-D-glucoside (skimming). These compounds were obtained from the methanolic extract of the seeds since this extract gave the best result in the MTT assay. All the three (3) isolated compounds were tested in the CAOV-4 human ovarian cancer cells and the MTT assay was performed at the 24, 48, and 72-hour time points. Following exposure of these cells for 2 days, the IC50 values for the 197 μM, 254 μM, and 216 μM concentrations respectively were 99.7, 87.3, and 70.03 $\mu g.mL^{-1}$. Based on the comet assay, exposure of CAOV-4 cells for 48 hours all the three (3) compounds exhibited double-strand breaks (indicative of possible interaction of these compounds with DNA). The toxicity and DNA damaging potential were correlated with the percentage of FITC-labeled annexin V cells. Diglucosyl caffeoyl ester caused cell death by apoptosis. The mode of cell demise was attributed to being due to necrosis in the case of 4-O-β-D-glucopyranosylcaffeic acid as well as Umbelliferone 7-O-β-D-glucoside (skimming) (Eskandani et al. 2014).

The seeds of *Descurainia sophia* (Brassicaceae (Cruciferae) family) have been reported to be part of the Korean medicine system. The ethanolic extract of *Descurainia sophia* seeds (EEDS) has ingredients that have cytotoxic (helveticoside) and anti-inflammatory potential (quercetin and syringaresinol) and this finding provided the researchers a basis for further evaluating its anticancer/cell death potential in suitable cell culture-based model systems. The involvement of the extrinsic pathway in augmenting cell death was demonstrated by this seed extract sensitizing A549 cells to TRAIL-mediated cell death. This augmentation of cell death was mediated, in major part, by the upregulation of the death receptors DR4 and DR5, at both the mRNA and protein levels in the TRAIL refractory A549 cells. This increased cell death in A549 cells was demonstrated based on combination treatment involving exposure to a certain concentration of EEDS and exogenously administered TRAIL. The involvement of DR5 was further verified by inhibiting this increase in cell death following a decrease in the CCAAT/enhancer-binding protein homologous protein (CHOP). This provided evidence for the involvement of its transcription in EEDS-mediated upregulation of DR5 (Park et al. 2016).

The plethora of published data on Grape Seed Extracts (GSE) and the bioactive components contained therein has necessitated the inclusion of the salient findings with respect to their cell death induction potential as well as details with respect to the model system and the methodologies adopted (Table 1).

CHALLENGES AND FUTURE DIRECTIONS

Extraction and Solvent System

The techniques selected to extract bioactive principles from

Table 1: Grape seed extracts and key findings *in vitro* and *in vivo.*

S.No.	Extract/ Compound Name	Cell Line/Animal Model	Concentration range- IC 50/ Treatment time	Major Findings	Reference
1	GSPs	HeLa, SiHa	0-100 μg.mL^{-1} 40 and 80 μg.mL^{-1}	Dose-dependent decrements and in the mitochondrial membrane potential increase in Bak-1 and decrease the level of Bcl-2 protein was seen in both cell lines and other apoptotic	Chen et al. (2014)
2	GSPs	Ca9-22 and HGF-1	50-200 μg.mL^{-1} IC50-150 μg.mL^{-1}	5-27% in sub G1;14-73% Annexin V positive; 2-63% DCFH-DA ROS level and decrease of 100-15% decrease of Rh123; 3-36% Increase of phosphorylated γH2AX protein (marker for double strand breaks)	Yen et al. (2015)
3	RSV-GSE	8 months old AOM-induced rodent Model	0.12% W/W of drug fed after 16 Weeks after 6week subcutaneous injection of AOM	50% decline in tumor with no GI toxicity compared with Sulindac ↓ in Nuclear β-catenin correlated with c-myc and cyclin D1, ΔΨɱ (18%), P53, Bax cleaved PARP and Cyt C & ther was concomitant ↓ in 50% CSCs, Bcl-2. Experimental manipulation of the p53 levels -a shRNA system -involvement of this protein in regulating Cox2 levels and not on RSV-GSE-mediated decrements -β-catenin; c-myc and cyclin-D1 levels.	Reddivari et al. (2016)
4	GSE/ GSPs/ (+)-catechin and (-)-epicatechin with oligomers being predominant	BIU87	0-200 μg.mL^{-1}	Dose-dependent toxicity; 48%-79% PI-based G1 block, Increase in Cyclin D1 & CDK4; 9-41% apoptotic cells and hyper condensed cells and fragmented nuclei was seen in 24h treatment. Survivin levels decreased and caspase-3 expression was elevated in a dose-dependent manner.	Liu et al. (2015)
5	GSPs	Tca8113	0-200 μg.mL^{-1} IC 50 of 24, 48 & 72 hours was 86.36, 43.65 and 31.17 μg.mL^{-1} respectively	Dose-dependent toxicity, 3-47% increased apoptosis, 28-76% migratory capabilities, and MMP-2 and MMP-9; also increase in Bax and decrease in Bcl-2. Phosphorylation of Akt as well as NF-κB activation and translocation to the nucleus was inhibited.	Yang et al. (2017)
6	Aqueous GSE	A431	50-200 μg.mL^{-1} IC50-111.11 μg.mL^{-1}	Dose-dependent toxicity, roundedness, irregular and cytoplasmic vacuolation of cells; Increase in ROS, 16 % apoptotic cell death, while 80.6% of them were eliminated by secondary necrosis. IC 50 of seed extract 60% loss in ΔΨɱ, 49% apoptotic, and 48% necrotic cells at the 24-hour time point.	Nirmala et al. (2018)
7	GSPs/RSV	MCF-7& MDA MDB-231	GSP-20, 40 μg.mL^{-1}, RES-10, 20 μM.ml^{-1}	The combination of RSV and GSP shows a significant difference when compared with a parental group, and an increase in Bax and decreased Bcl-2 was seen in both cell lines. At 20μg.mL^{-1} GSP + 10mM RSV decrease the DNMT and HDAC activity	Gao & Tollefsbol (2018)
8	GSPs/GSE	ECA109	0-400 μg.mL^{-1}. IC50 12h-66.442±13.54, 24h-51.713±12.69, 48h-37.158±13.07	Higher the dose and time of GSPE 80μg.mL^{-1} for 48h - higher the inhibition of IL-6, and COX-2 and with BAY11-7082 is stronger inhibition when compared when treated alone; and also ↓ of IKK, IkB, p-IkB in dose-dee pendent and↑of mRNA and protein expression of p50, and p65 and BAY-11 alone there is no decrease in IKK and mRNA levels.	Guo et al. (2018)

Table cont...

S.No.	Extract/ Compound Name	Cell Line/Animal Model	Concentration range- IC 50/ Treatment time	Major Findings	Reference
9	GSP B2	HT29 and LoVo cells	0-200 μg.mL^{-1} HT29 IC50=15μM and LoVo IC50=12μM at 48 hrs.	Procyanidin (6, 12, 24μM) shows a dose-dependent increase in the number of the autophagosome, high expression GPF-LC3, Beclin1, LCII, and Agt5 - and expression of LC3I was inhibited. 1-12% - ↑apoptosis and upregulation of Bax, Caspase-3, and ↓ Bcl-2. 3MA reversed the inhibition of apoptosis and concluded the inhibition of autophagy reduction leads to anti-apoptosis	Zhang et al. (2019)
10	GSP	NSCLC-A549& H23; SCLC-DMS114 & pre-malignant 1198	0-30 μg.mL^{-1}	GSE shows a dose-dependent increase in 6-keto PGF1α and 15HETE inhibition of (COX-2)/prostaglandin E2 (PGE2) eicosanoid pathways.	Mao et al. (2016)
11	GS Catechin and Epicatechin	*In vivo* –HCC animal model	25,50,100 mg.kg^{-1}	GS shows ↓ pre-neoplastic foci formation; 4&10fold decrease respectively in the number and the area of GST-P in the livers of treated rats. GSEs effects-liver: ↑ apoptosis induction; ↓ cell proliferation, oxidative stress, and HDAC activity and inflammation markers (COX-2, iNOS, NF-κB -p65 and p- TNF receptor expression).	Hamza et al. (2018)
12	GSP	H441	0-10 μg.mL^{-1}	Plain SLN is non-toxic. They show dose-dependent, high bioavailability, and GSE-loaded SLN - antioxidant effect for a longer duration than free GSE indicative of controlled release of their payload.	Castellani et al. (2018)
13	GS-GA, CAT, epi-CAT	MCF-7	0.05–100 μg.mL^{-1}	Higher GJIC –alteration in the functionality of the Connexin proteins already present in the cells and/or the opening of the gap shut abnormally. Immunolocalization of the Cx43 protein in MCF-7 cells -treated with different GSE concentrations (2 h or 24 h)	Leone et al. (2019)
14	GSP B2	*In vivo*- CCl4-induced mouse Liver model	150 mg.kg^{-1}	HSCs *in vivo* and *in vitro* -↓ VEGF-A, HIF-1α, α-SMA, Col-1, and TGF-β1; SAG and cyclopamine –proved PB2 targets in the Hh pathway	Feng et al. (2019)
15	GSPs	A375 & Hs294t	0-60 μg.mL^{-1}	↑-Dose-dependent toxicity, 12-30% apoptosis, downregulation of Bcl-2, Bc-x1 and and β-Catenin. GSPs (0.2 and 0.5%, w/w) supplemented with AIN76A (control diet) –xenograft Model -greatly inhibited the growth of melanoma cells and decreased the growth of Mel928 (β-catenin-activated), did not inhibit the xenograft growth of Mel1011 (β-catenin-inactivated) cells.	Vaid et al. (2016)
16	GSE	HNSCC- Detroit 562 and FaDu cells		GSE activated AMP-activated protein kinase (AMPK) and decreased Akt/mTOR/4E-BP1/S6K signaling in both Detroit 562 and FaDu cells. FaDu xenograft tumor model -GSE feeding of nude mice –activation of AMPK and autophagy induction	Shrotriya et al. (2015a)
17	GSE& RSV	4NQO-induced tongue cancer in C57BL/6	100 μg.mL^{-1}	GSE and Res –multiple possible mechanisms –prophylactic efficacy -pleiotropic effects on cell proliferation, apoptosis, cellular metabolism, and autophagy	Shrotriya et al. (2015b)

GSPs- Grape seed Procyanidins, GSE- Grape seed extract, RSV- Resveratrol, IC50- 50% of cell growth inhibitory concentration, AOM- Azoxymethane, GI- Gastrointestinal, ΔΨm -Mitochondrial membrane potential, DNMT- DNA methyltransferase, HDAC- Histone deacetylases, 3-MA- 3-Methyladenine, COX-2- cyclooxygenase-2, (PGE2)- Prostaglandin E2, iNOS- inducible Nitric oxide synthase, NF-κB- nuclear factor-kappa B, p-TNF- phosphorylated tumor necrosis factor receptor, GST-P- placental Glutathione-S-transferase, GJIC- gap-junction-mediated cell-cell communications, Hh-Hedgehog pathway, SAG- Smoothened agonist, HSC-Hepatic stellate cell lines, αmSMA- Smooth muscle actin, Col-1-Collagen type 1, VEGF-A –Vascular endothelial growth factor A, 4NQO- 4-nitroquinoline-1- oxide.

seeds as well as their optimization is a pivotal first step for succeeding in developing a drug with chemotherapeutic potential. Different techniques were employed to extract and remove the compounds from seed extracts. The chemical (solvent-based) extraction and classical Soxhlet, maceration, distillation, and physical-based methods like cold press extraction technique is widely used to extract the compounds. The major drawbacks of this technique were its time-consuming nature as well as the volume of organic solvents used (De Castro & Garcia 1998). The green extraction method has been used extensively in the past 2 decades. These methods include advanced techniques like ultrasound, microwave, and supercritical CO_2 extraction, used singly or in combination, which can be optimized for the maximal extraction of bioactive active compounds from seeds (Casazza et al. 2010, Chemat et al. 2012, Singh et al. 2014, Zhang et al. 2018). Sometimes seeds may be requiring additional steps (pulverization, pre-leaching methods), which facilitate the subsequent extraction of compounds from the seed matrix (Bimakr et al. 2013, Dhobi et al. 2009, Dabas et al. 2019).

Synergistic effect of seed extracts when combined with two or more compounds basis for crude seed extract-based research for drug development potential

Despite some factors, such as seasonal variations that may impact the levels of bioactive components in the seeds as well as seed-to-seed variability, crude extracts have potential in terms of their anti-cancer efficacy. Once the extraction process is optimized (Please see Fig. 1 for the experimental flow that can be adopted as a teaching and research tool for the systematic extraction of bioactive principles from seed and other plant sources), these extracts should be tested in suitable *in vitro* and *in vivo* cancer models. Reproducibility in the therapeutic effect warrants optimization of the efficacy of the formulation involving these extracts. Also, toxicity evaluation needs to be done in drug development involving crude extracts from seed sources. Also, in certain cases, the crude extracts may synergize with currently available chemotherapeutic drugs and are elaborated upon below with suitable examples.

A study showed that Taxol (paclitaxel) is a powerful anti-cancer drug widely used against several types of malignant tumors. The effects of Taxol-encapsulated liposomes (T) alone and in combination between Eruca sativa seed extract on nuclear factor kappa B (NF-κB), cyclooxygenase-2 (COX-2), and B-cell lymphoma-2 (Bcl-2) gene expression levels were investigated in rat mammary gland carcinogenesis induced by 7,12 dimethylbenz(α) anthracene (DMBA)

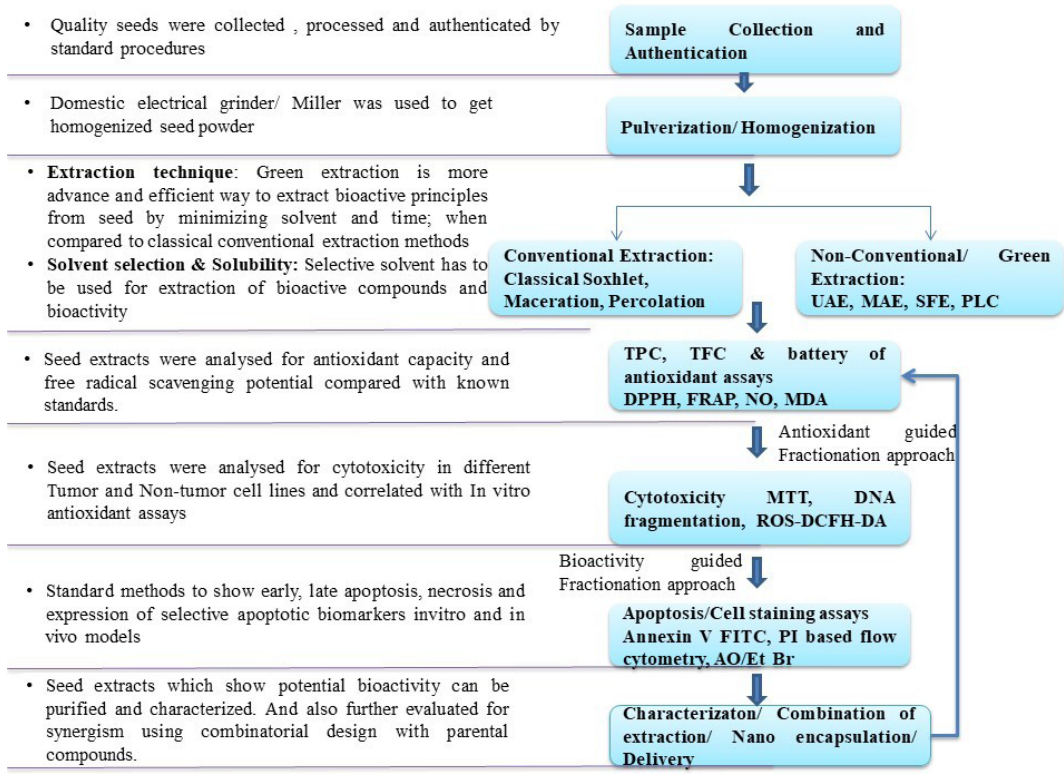

Fig. 1: Schematic representation of steps involved in the identification of apoptotic potential of seed extracts.

using qRT-PCR. Results showed that DMBA increased NF-κB, COX-2, and Bcl-2 gene expression levels and lipid peroxidation (LP) while decreasing glutathione-S-transferase (GST) and superoxide dismutase (SOD) activities and total antioxidant concentration (TAC) compared to the control group. T and T-SE treatment reduced NF-κB, COX-2, and Bcl-2 gene expression levels and LP. Hence, T and T-SE treatment appeared to reduce inflammation and cell proliferation, while increasing apoptosis, GST and SOD activities, and TAC (Shaban et al. 2016). A study showed the synergistic effects of GSPs and Res on inhibiting MDA-MB-231 and MCF-7 human breast cancer cells. Our results of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays and clonogenic assays indicate that treatments with the combinations of GSPs and Res synergistically decreased cell viability and posttreatment cell proliferation in both cell lines (Gao et al. 2018). Chavoshi et al. (2017) showed that a combination of silibinin (a molecule extractable from a seed source), paclitaxel, and cisplatin show a synergistic effect, in MCF-7 breast cancer cells than when treated alone (Chavoshi et al. 2017). A 70% ethanolic extract of *Blepharis persica* seed combined with Doxorubicin induces toxicity, apoptosis, necrosis, downregulation of Bcl2, and upregulation of Bax protein in the HT29 cell line (Aghaabbasi et al. 2020). Czajkowska et al. (2017) reported that the novel octahydropyrazino[2,1-a:5,4-a']di-isoquinoline derivative combined with *Nigella sativa* showed a synergistic effect in terms of cell toxicity, apoptosis, and mitochondrial membrane potential (Czajkowska et al. 2017). A study evaluated the anticancer potential of methanolic extracts of Berberis

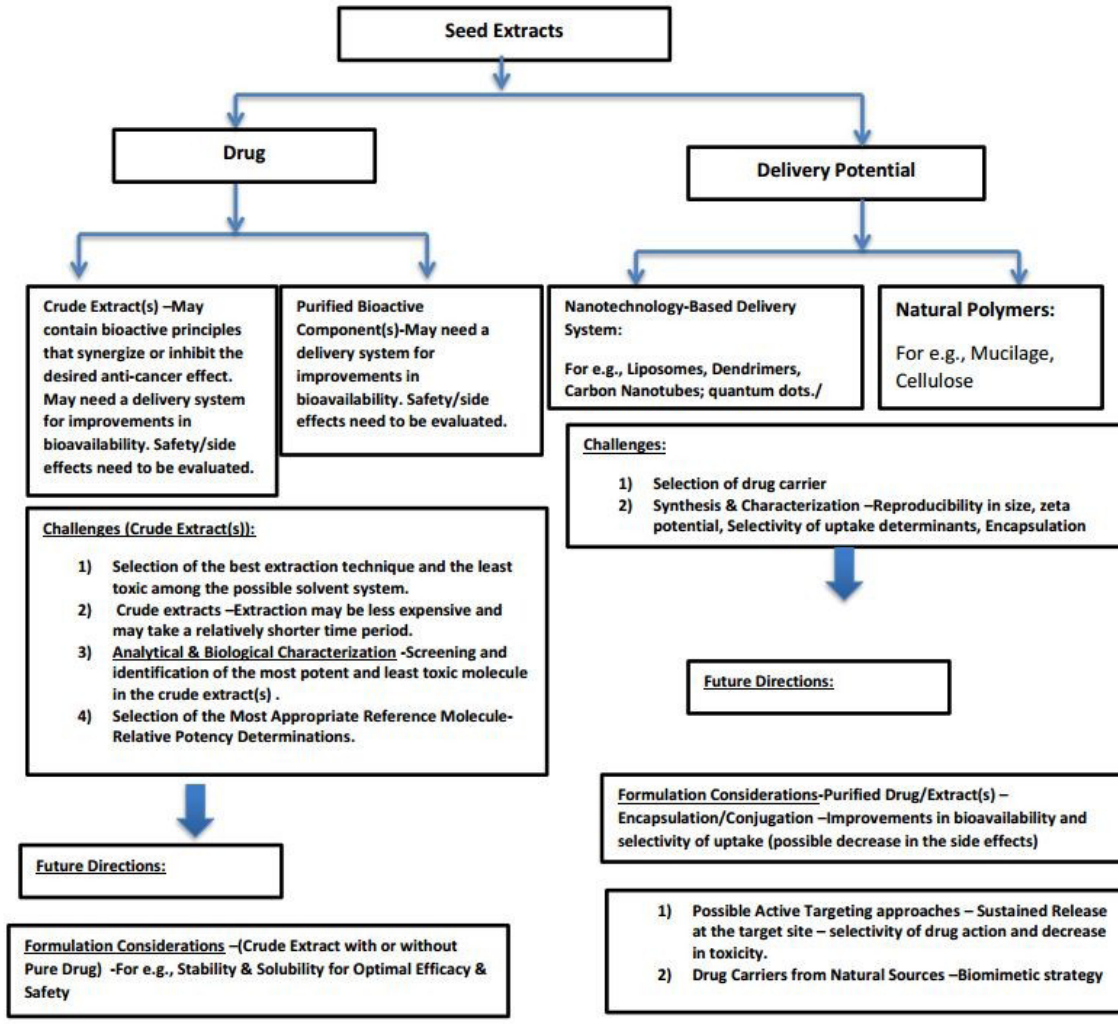

Fig. 2: Experimental flow for seed extracts/ or compounds as drug/delivery developmental system.

aristata root and Azadirachta indica seeds prepared by various extraction techniques in human osteosarcoma (HOS) cells. Soxhlation extract of B. aristata (BAM-SX) and sonication extract of *Azadirachta indica* (AIM-SO) were most effective in inducing apoptosis in parental drug-sensitive, as well as resistant cell type developed by repeated drug exposure. (27.82 $\mu g.ml^{-1}$ and 28.65 $\mu g.ml^{-1}$) toxicity (Sengupta et al. 2017).

These aforesaid promising findings provide a sound rationale for the need to delineate the best bioactive principles or a combination thereof,that can possibly enhance the anti-cancer potential of currently available synthetic drugs as well as those of natural origin. Also, Fig. 2 provides a strategy for the encapsulation and subsequent increments in uptake and the subsequent cell death potential of seed extracts singly and/or in combination with other molecules (natural or synthetic in origin).

CONCLUSIONS

Our discussion clearly provides evidence of the beneficial apoptotic effects of seed extracts from diverse sources in different *in vitro* and *in vivo* model systems. Also, this review highlights the need to perform an HTS-based screening strategy to short-list the major seed sources, thereby hastening the drug development process. Moreover, this review underscores the need for the inter-laboratory validation of protocols as well as for defining certain variables, like the dormancy status of the seeds. Furthermore, this design can be extended to identifying specific seed extract-drug combinations that can reliably synergize and augment cell death.

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ABBREVIATIONS

GSP:grape seed proanthocyanidins, HTS:High throughput screening, Annexin V-FITC/PI: Annexin A5-Fluorescein isothiocyanate/ propidium iodide, FDA: US Food and Drug administration, PARP: Poly (ADP-ribose) polymerase, WIF1: WNT Inhibitory Factor 1, ALDH1A1:Aldehyde Dehydrogenase 1 Family Member A1, Dclk1: Doublecortin Like Kinase1,EZH2: Enhancer of zeste homolog 2 ,MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromidefor, TEM: Transmission electron microscope, $DiOC_6$: Ionic fluorochrome, 3,3′-dihexyloxacarbocyanine iodide, DCFH-DA: 2′,7′-Dichlorofluorescin Diacetate, TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand, CCAAT: enhancer-binding protein delta (CEBPD), DLBCL: Diffuse large B-cell lymphoma, AR: Androgen Receptor, PSA: Prostate Specific Antigen, TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling, AKT: Protein kinase B, GSK3β: Glycogen synthase kinase 3 beta, SLN: solid lipid nanoparticles, RES: Resveratrol, GST: Glutathione S-transferases , SOD: Superoxide dismutase, DMBA: 7,12 dimethylbenz(α) anthracene, OX26 mAb: anti-transferrin receptor monoclonal antibody.

REFERENCES

Aghaabbasi, K., Askari, N., Kumleh, H.H., Torkzadeh-Mahani, M. and Ramzani-Ghara A. 2020. The *Blepharis persica* seed hydroalcoholic extract synergistically enhances the apoptotic effect of doxorubicin in human colon cancer and gastric cancer cells. Mol, Biol. Rep., 47(2): 843-853.

Ahmed, I., Roy, B.C., Subramaniam, D., Ganie, S.A., Kwatra, D., Dixon, D., Anant, S., Zargar, M.A. and Umar, S. 2016. An ornamental plant targets epigenetic signaling to block cancer stem cell-driven colon carcinogenesis. Carcinogenesis, 37(4): 385-396.

Ali, A.Q., Farah, M.A., Abou-Tarboush, F.M., Al-Anazi, K.M., Ali, M.A., Lee, J., Hailan, W.A. and Mahmoud, A.H. 2019. Cytogenotoxic effects of *Adenium obesum* seeds extract on breast cancer cells. Saudi J. Biol. Sci., 26(3): 547-553.

Al-Mamun, M.A., Husna, J., Khatun, M., Hasan, R., Kamruzzaman, M., Hoque, K.M.F., Reza, M.A. and Ferdousi, Z. 2016. Assessment of antioxidant, anticancer and antimicrobial activity of two vegetable species of Amaranthus in Bangladesh. BMC Compl. Altern. Med., 16(1): 1-11.

Banjerdpongchai, R., Wudtiwai, B., Khaw-On, P., Rachakhom, W., Duangnil, N. and Kongtawelert, P. 2016. Hesperidin from Citrus seed induces human hepatocellular carcinoma HepG2 cell apoptosis via both mitochondrial and death receptor pathways. Tumor Biol., 37(1): 227-237.

Bimakr, M., Rahman, R.A., Saleena Taip, F., Adzahan, N.M. and Islam Sarker, Z. 2013. Ultrasound-assisted extraction of valuable compounds from winter melon (*Benincasa hispida*) seeds. Int. Food Res. J., 20(1).

Casazza, A.A., Aliakbarian, B., Mantegna, S., Cravotto, G. and Perego, P. 2010. Extraction of phenolics from *Vitis vinifera* wastes using non-conventional techniques. Journal of Food Engineering, 100(1): 50-55.

Cassiem, W. and de Kock, M. 2019. The anti-proliferative effect of apricot and peach kernel extracts on human colon cancer cells *in vitro*. BMC Compl. Altern. Med., 19(1):1-12.

Castellani, S., Trapani, A., Spagnoletta, A., Di Toma, L., Magrone, T., Di Gioia, S., Mandracchia, D., Trapani, G., Jirillo, E. and Conese, M. 2018. Nanoparticle delivery of grape seed-derived proanthocyanidins to airway epithelial cells dampens oxidative stress and inflammation. J. Transl. Med., 16(1): 1-15.

Chavoshi, H., Vahedian, V., Saghaei, S., Pirouzpanah, M.B., Raeisi, M. and Samadi, N. 2017. Adjuvant therapy with silibinin improves the efficacy of paclitaxel and cisplatin in MCF-7 breast cancer cells. Asian Pac. J.

Cancer Prev., 18(8): 2243.
Chemat, F., Vian, M.A. and Cravotto, G. 2012. Green extraction of natural products: concept and principles. Int. J. Mol. Sci., 13(7): 8615-8627.
Chen, Q., Liu, X.F. and Zheng, P.S. 2014. Grape seed proanthocyanidins (GSPs) inhibit the growth of cervical cancer by inducing apoptosis mediated by the mitochondrial pathway. PLoS One, 9(9): 107045.
Chene, G., Baillif, V., Van Goethem, E., Branka, J.E., Ionescu, T., Robert, G. and Lefeuvre, L. 2015. *In vitro* and *in vivo* anti-inflammatory effect of a biotechnologically modified borage seed extract: Evidence for lipid pro-resolving mediators' implication in the enhancement of psoriatic and atopic dermatitis lesions. J. Cosmet. Dematol. Sci. Appl., 5(02): 151.
Ciftci, G.A., Işcan, A. and Kutlu, M. 2015. Escin reduces cell proliferation and induces apoptosis on glioma and lung adenocarcinoma cell lines. Cytotechnology, 67(5): 893-904.
Czajkowska, A., Gornowicz, A., Pawlowska, N., Czarnomysy, R., Nazaruk, J., Szymanowski, W., Bielawska, A. and Bielawski, K. 2017. Anticancer Effect of a Novel Octahydropyrazino [2, 1-a: 5, 4-a′] diisoquinoline Derivative and Its Synergistic Action with *Nigella sativa* in Human Gastric Cancer Cells. BioMed Res. Int., 2017.
Dabas, D., Elias, R.J., Ziegler, G.R. and Lambert, J.D. 2019. *In vitro* antioxidant and cancer inhibitory activity of a colored avocado seed extract. Int. J. Food Sci., 2019: 63-84.
Davatgaran-Taghipour, Y., Masoomzadeh, S., Farzaei, M.H., Bahramsoltani, R., Karimi-Soureh, Z., Rahimi, R. and Abdollahi, M. 2017. Polyphenol nanoformulations for cancer therapy: Experimental evidence and clinical perspective. Int. J. Nanomed., 12: 2689.
De Castro, M.L. and Garcıa-Ayuso, L.E. 1998. Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. Anal. Chim. Acta, 369(1-2): 1-10.
Dhobi, M., Mandal, V. and Hemalatha, S. 2009. Optimization of microwave-assisted extraction of bioactive flavonolignan-silybinin. J. Chem. Metrol. 3(1).
Eskandani, M., Dadizadeh, E., Hamishehkar, H., Nazemiyeh, H. and Barar, J. 2014. Geno/cytotoxicity and apoptotic properties of phenolic compounds from the seeds of *Dorema glabrum* Fisch. CA. BioImpacts-BI, 4(4): 191.
Feng, J., Wang, C., Liu, T., Li, J., Wu, L., Yu, Q., Li, S., Zhou, Y., Zhang, J., Chen, J. and Ji, J. 2019. Procyanidin B2 inhibits the activation of hepatic stellate cells and angiogenesis via the Hedgehog pathway during liver fibrosis. J. Cell. Mol. Med., 23(9): 6479-6493.
Gao, Y. and Tollefsbol, T.O. 2018. Combinational proanthocyanidins and resveratrol synergistically inhibit human breast cancer cells and impact epigenetic–mediating machinery. Int. J. Mol. Sci., 19(8): 2204.
Guo, F., Hu, Y., Niu, Q., Li, Y., Ding, Y., Ma, R., Wang, X., Li, S. and Xie, J. 2018. Grape seed proanthocyanidin extract inhibits human esophageal squamous cancerous cell line eca109 via the nf-κb signaling pathway. Medicinski Arhiv., 2018: 56-71
Hamza, A.A., Heeba, G.H., Elwy, H.M., Murali, C., El-Awady, R. and Amin, A. 2018. Molecular characterization of the grape seeds extract's effect against chemically-induced liver cancer: *In vivo* and *in vitro* analyses. Sci. Rep., 8(1): 1-16.
Lakshmi, T., Ezhilarasan, D., Nagaich, U. and Vijayaragavan, R. 2017. *Acacia catechu* ethanolic seed extract triggers apoptosis of SCC-25 cells. Pharmacogn. Mag., 13*:* S405.
Leone, A., Longo, C., Gerardi, C. and Trosko, J.E. 2019. Pro-apoptotic effect of grape seed extract on MCF-7 involves the transient increase of gap junction intercellular communication and Cx43 up-regulation: A mechanism of chemoprevention. Int. J. Mol. Sci., 20(13): 3244.
Liu, J., Zhang, W.Y., Kong, Z.H. and Ding, D.G. 2016. Induction of cell cycle arrest and apoptosis by grape seed procyanidin extract in human bladder cancer BIU87 cells. Eur. Rev. Med. Pharmacol. Sci., 20(15): 3282-3291.
Liu, Y., Ma, S.S., Ibrahim, S.A., Li, E.H., Yang, H. and Huang, W. 2015. Identification and antioxidant properties of polyphenols in lotus seed epicarp at different ripening stages. Food Chem., 185: 159-164.
Mahli, A., Koch, A., Czech, B., Peterburs, P., Lechner, A., Haunschild, J., Müller, M. and Hellerbrand, C. 2015. Hepatoprotective effect of oral application of a silymarin extract in carbon tetrachloride-induced hepatotoxicity in rats. Clin. Phytoscience, 1(1): 1-8.
Mao, J.T., Smoake, J., Park, H.K., Lu, Q.Y. and Xue, B. 2016. Grape seed procyanidin extract mediates antineoplastic effects against lung cancer via modulations of prostacyclin and 15-HETE eicosanoid pathways. Cancer Prev. Res., 9(12): 925-932.
Nirmala, J.G., Celsia, S.E., Swaminathan, A., Narendhirakannan, R.T. and Chatterjee, S. 2018. Cytotoxicity and apoptotic cell death induced by *Vitis vinifera* peel and seed extracts in A431 skin cancer cells. Cytotechnology, 70(2): 537-554.
Ogaly, H.A., Eltablawy, N.A., El-Behairy, A.M., El-Hindi, H. and Abd-El-salam, R.M. 2015. Hepatocyte growth factor mediates the antifibrogenic action of *Ocimum bacilicum* essential oil against CCl4-induced liver fibrosis in rats. Molecules, 20(8): 13518-13535.
Park, J.S., Lim, C.J., Bang, O.S. and Kim, N.S. 2016. Ethanolic extract of *Descurainia sophia* seeds sensitizes A549 human lung cancer cells to TRAIL cytotoxicity by upregulating death receptors. BMC Compl. Altern. Med., 16(1): 1-8.
Reddivari, L., Charepalli, V., Radhakrishnan, S., Vadde, R., Elias, R.J., Lambert, J.D. and Vanamala, J.K. 2016. Grape compounds suppress colon cancer stem cells *in vitro* and a rodent model of colon carcinogenesis. BMC Compl. Altern. Med., 16(1): 1-12.
Seca, A.M. and Pinto, D.C. 2018. Plant secondary metabolites as anticancer agents: successes in clinical trials and therapeutic application. Int. J. Mol. Sci., 19(1): 263.
Sengupta, P., Raman, S., Chowdhury, R., Lohitesh, K., Saini, H., Mukherjee, S. and Paul, A. 2017. Evaluation of apoptosis and autophagy inducing potential of *Berberis aristata*, *Azadirachta indica*, and their synergistic combinations in parental and resistant human osteosarcoma cells. Front. Oncol., 7: 296.
Shaban, N., Abdel-Rahman, S., Haggag, A., Awad, D., Bassiouny, A. and Talaat, I. 2016. The combination between taxol-encapsulated liposomes and *Eruca sativa* seed extract suppresses mammary tumors in female rats induced by 7, 12 Dimethylbenz (α) anthracene. Asian Pac. J. Cancer Prev., 17(1): 117-123.
Shrotriya, S., Deep, G., Lopert, P., Patel, M., Agarwal, R. and Agarwal, C. 2015a. Grape seed extract targets mitochondrial electron transport chain complex III and induces oxidative and metabolic stress leading to cytoprotective autophagy and apoptotic death in human head and neck cancer cells. Mol. Carcinog., 54(12): 1734-1747.
Shrotriya, S., Tyagi, A., Deep, G., Orlicky, D.J., Wisell, J., Wang, X.J., Sclafani, R.A., Agarwal, R. and Agarwal, C. 2015b. Grape seed extract and resveratrol prevent 4-nitroquinoline 1-oxide induced oral tumorigenesis in mice by modulating AMPK activation and associated biological responses. Mol. Carcinog., 54(4): 291-300.
Singh, M., Jha, A., Kumar, A., Hettiarachchy, N., Rai, A.K. and Sharma, D. 2014. Influence of the solvents on the extraction of major phenolic compounds (punicalagin, ellagic acid and gallic acid) and their antioxidant activities in pomegranate aril. J. Food Sci. Technol., 51(9): 2070-2077.
Vaid, M., Singh, T., Prasad, R. and Katiyar, S.K. 2016. Bioactive proanthocyanidins inhibit growth and induce apoptosis in human melanoma cells by decreasing the accumulation of β-catenin. Int. J. Oncol., 48(2): 624-634.
Yang, N., Gao, J., Cheng, X., Hou, C., Yang, Y., Qiu, Y., Xu, M., Zhang, Y. and Huang, S. 2017. Grape seed proanthocyanidins inhibit the proliferation, migration and invasion of tongue squamous cell carcinoma cells through suppressing the protein kinase B/nuclear factor-κB signaling

pathway. Int. J. Mol. Med., 40(6): 1881-1888.

Yen, C.Y., Hou, M.F., Yang, Z.W., Tang, J.Y., Li, K.T., Huang, H.W., Huang, Y.H., Lee, S.Y., Fu, T.F., Hsieh, C.Y. and Chen, B.H. 2015. Concentration effects of grape seed extract in anti-oral cancer cells involving differential apoptosis, oxidative stress, and DNA damage. BMC Complem. Altern. Med., 15(1): 1-9.

Zhang, Q.W., Lin, L.G. and Ye, W.C. 2018. Techniques for extraction and isolation of natural products: A comprehensive review. Chinese Med., 13(1): 1-26.

Zhang, R., Yu, Q., Lu, W., Shen, J., Zhou, D., Wang, Y., Gao, S. and Wang, Z. 2019. Grape seed procyanidin B2 promotes autophagy and apoptosis in colorectal cancer cells via regulating PI3K/Akt signaling pathway. OncoTargets Ther., 12: 4109.