Research Paper

In Vitro Evaluation of *Brassica* Sprouts for Antioxidant and Antiproliferative Potential

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The present study was conducted on sprouts (5 and 7 days) of turnip (*Brassica rapa*), cauliflower (*Brassica oleracea*) and mustard (*Brassica juncea*) for bioactivity. Their antioxidant potential was assessed by 2,2-diphenyl-1-picrylhydrazyl, superoxide anion radical scavenging assays at 0.125-2 mg/ml concentration. The extract of turnip, cauliflower and mustard (5 and 7 days) showed a potent antioxidant effect and significant cytotoxic effect at 100 µg/ml concentration of extract. The antiproliferative potential was also evaluated by applying cell cycle and intracellular reactive oxygen species generation assay at IC_{50} value. Various phytochemicals and hydrolytic products of glucosinolates were observed in a different extract of turnip, cauliflower, and mustard sprouts. Flow cytometric analysis showed that all the extracts caused an increase in a G_0 population of the PC-3 cells in cell cycle analysis and an increase in intracellular reactive oxygen species generation as compared to untreated cells. Confocal imaging of the cells stained with 4',6-diamidino-2-phenylindole and 2,7-dichlorodihydrofluorescein diacetate showed DNA fragmentation and increase of fluorescence which supports apoptosis and intracellular generation of the reactive oxygen species as the possible cause of cell death. The reported activity was correlated with the presence of different organosulfur compounds identified by gas chromatography-mass spectroscopy.

Key words: *Brassica* sprout, antioxidant assay, cell cycle, ROS, confocal microscopy, MTT assay

In recent years, health protection by natural products or plant derived foods has received a considerable attention[1]. Specific groups of vegetables are particularly rich in potentially protective phytochemicals, especially *Brassica* contains a high concentration of constituents with antioxidant properties (e.g. carotenoids, vitamin C and folates) as well as glucosinolates precursors of isothiocyanates (ITCs) and indoles that modulate the activity of xenobiotic biotransformation[2]. A diet rich in fruits, cruciferous vegetables has been linked to reduce the risk of many chronic diseases, including cancer. The importance of this family for food crops has led to its selective breeding throughout the history. Cruciferous sprouts of broccoli, alfalfa, buckwheat and bean etc. have received considerable attention due to their rich content of health-promoting phytochemical constituents such as glucosinolates phenolic compounds and ascorbic acid related to cancer prevention as well as having antioxidant properties[3-7].

During sprouting generally, phytonutrient content increases as compared to seeds and consumption of these sprouts is the best way to gain all the health benefits[8,9]. Naturally occurring ITCs, found abundantly in cruciferous vegetables, inhibit tumorigenesis by inducing apoptosis and arresting cell cycle progression. In addition, induction of detoxification enzymes suggested the mechanism through which cruciferous vegetable consumption or the consumption of the glucosinolate breakdown products reduces cancer risk[10]. Due to these protective effects, consumers can find an extraordinary variety of different types of sprouts which represent cruciferous family in the market. The use of seed sprouts as food has spread in the past few decades from far eastern countries to parts of the western world. Therefore, the objectives of the current study are to investigate different extracts for the antioxidant and antiproliferative activity of 5 and 7 days old sprouts from cauliflower, turnip and mustard. In addition, volatile phytoconstituents were also identified by gas chromatography-mass spectroscopy

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(GC-MS). Moreover, the phytoconstituents of various extracts were examined in order to study their possible relationship with antioxidant and antiproliferative studies.

MATERIALS AND METHODS

Roswell Park Memorial Institute (RPMI-1640) medium, fetal bovine serum (FBS), penicillin, streptomycin, L-glutamine, 4',6-diamidino-2-phenylindole (DAPI) and the fluorescent probes 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA), 2,2'-diphenyl-1-picryl hydrazyl (DPPH), ribonuclease (RNase) A, and Triton X-100 were obtained from Sigma-Aldrich Corp, St. Louis, MO, USA. Propidium iodide (PI) was purchased from Invitrogen Life Technologies (Carlsbad, CA). Ammonium molybdate, ethylenediaminetetraacetic acid (EDTA), nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), nicotinamide adenine dinucleotide phosphate reduced (NADH), dimethyl sulfoxide (DMSO), ascorbic acid and remaining reagents were of analytical grade. A prostate cancer (PC-3) cell line was obtained from Indian Institute of Integrative Medicine (IIIM), Jammu, India.

Plant material:

The seeds of cauliflower (*B. oleracea*), turnip (*B. rapa*) and mustard (*B. juncea*) were procured from Amritsar local market. Seeds were surface sterilized with 70% ethanol for 1 min followed by 1.3% sodium hypochlorite for 15 min and then allowed to germinate in seed germinator. The homogenized plant material was labeled according to their harvested stage like 5 days of mustard (M5D), 7 days of mustard (M7D), 5 days of cauliflower (C5D), 7 days of cauliflower (C7D), 5 days of turnip (T5D) and 7 days of turnip (T7D). All sprouts were grown with a 16 h light and 8 h dark photoperiod at 24°, rapidly and gently collected from the trays and homogenized with a grinder.

Extraction procedure:

Extraction of plant material was done using the method described by Liang *et al.*[11] fresh plant material was homogenized in water for 5 min and left for autolysis at room temperature for 30 min After autolysis, the meal was extracted two times with dichloromethane (DCM), which was combined and then salted with 2.5 g anhydrous sodium sulfate and the fraction was dried at 30° under vacuum on a rotary evaporator.

DPPH radical scavenging assay:

The hydrogen donating or a radical scavenging ability of test samples was measured using the stable radical DPPH[12]. The reaction mixture contains 100 µl of different fraction concentrations (0.125 to 2 mg/ml) and 2 ml of DPPH (0.1 mM in methanol solution). The absorbance of the reaction mixture was recorded against the blank at 517 nm. The percent decoloration of the sample in DPPH assay was calculated as radical scavenging activity %=$(A_0-A_1/A_0)\times100$, where, A_0 is the absorbance of control (without test sample), A_1 is the absorbance of the reaction mixture (with test sample).

Superoxide anion radical scavenging assay:

The superoxide anion scavenging activity of different test samples was analyzed by using a method described by Nishikimi *et al.*[13] Different solutions like 1 ml of 156 µM NBT, 1 ml of 468 µM NADH prepared in phosphate buffer (100 mM, pH 7.4) and various concentration of test samples (0.125 to 2 mg/ml) were mixed and the reaction was started by adding 100 µl of PMS solution (60 µM) prepared in phosphate buffer (100 mM, pH 7.4). The reaction mixture incubated at 25° for 5 min and absorbance was recorded at 560 nm against the control. Radical scavenging activity %=$(A_0–A_1/A_0)\times100$, where A_0 is the absorbance of control, A_1 is the absorbance of a test sample.

Cell lines and culture:

The human PC-3 cell line grown and maintained in RPMI-1640 medium having FBS (10%), penicillin (100 units/ml), streptomycin (100 mg/ml), glutamine (2 mM) were maintained in CO_2 incubator at 37°, 90% humidity and 5% CO_2 conditions. The cells treated with extracts dissolved in DMSO while the untreated control cultures received only DMSO.

MTT assay:

The dimethylthiazolyldiphenyltetrazolium bromide (MTT) assay was performed following the well-described procedure with minor modifications[14]. Human PC-3 cells were centrifuged at 1000 rpm for 5 min at 4°. The cell pellet was resuspended in complete growth medium to get 1.5×10^5 cells/ml and 100 µl of cell suspension/well was seeded in tissue culture plate. Cells were treated with different concentrations of test material incubated for 12 h in a CO_2 incubator. Thereafter, 20 µl of freshly prepared MTT solution (5 mg/ml in PBS, sterile filtered) was added to each well and thoroughly mixed. The samples were incubated for 4 h at 37°, to allow metabolization of MTT. The supernatant was aspirated out after centrifuging at

2000 rpm for 10 min MTT-formazan crystals (MTT metabolic product) were resuspended in 100 µl of DMSO. Thereafter, plates were stirred for 20 min in order to dissolve formazan crystals and absorbance was measured at 570 nm.

Cell cycle analysis using propidium iodide:

PC-3 cells incubated in RPMI medium supplemented with 10% FBS in 24 well plates, at 1×10^6 cells/well with or without different sprouts extract at their respective IC_{50}. After 12 h of treatment, the floating and trypsinized cells were harvested and centrifuged at 1500 rpm for 5 min then washed with PBS twice. For each well, a volume of the cell suspension corresponding to 1×10^6 cells was centrifuged and the resultant cell pellet was resuspended in ice-cold PBS (1 ml). The cells were fixed in chilled 70% ethanol and incubated for 2 h. The supernatant was discarded and cells were suspended in 500 ml PBS with 15 ml propidium iodide (1 mg/ml). Cells were analyzed in FL-2 channel by BD Accuri C6 flow cytometer (BD Biosciences Immunocytometry Systems, San Jose, CA). DNA content and cell cycle phase distributions were modeled from events by excluding cell aggregates based on scatter plots[15].

Measurement of intracellular peroxides:

The cells plated in 24 well plates, at 1×10^6 cells allowed to adhere for 6 h and then grown in the presence or absence of extract. The cells were harvested after 12 h by collecting trypsinized cells together with floating cells in the medium and stained with DCFH-DA for 30 min at the end of treatment. The cell suspension corresponding to 1×10^6 cells was centrifuged and the resultant cell pellet was resuspended in ice-cold PBS (1 ml). A majority of cellular reactive oxygen species (ROS) were produced by mitochondria during stress conditions that disrupt mitochondrial electron transport. Therefore, a level of intracellular peroxides in PC-3 cells treated with extract at IC_{50} for 12 h was determined by using DCFH-DA[16].

GC-MS analysis:

The GC-MS analysis was carried out on a Shimadzu (QP 2010) series GC-MS (Tokyo, Japan), AOC-20i auto-sampler coupled and a DB-5MS capillary column, (30 mm×0.25 mm i.d, 0.25 µm). The initial temperature of the column was 70° maintained for 4 min programmed to 230° at 4°/min held for 15 min at 230°; the sample injection volume was 2 µl in GC grade DCM. Helium was used as carrier gas at a flow rate of 1.1 ml/min in split mode (1:50).

Statistical analysis:

The superoxide anion radical scavenging assay and DPPH assay was performed in triplicate and the data presented as mean. To compare the statistical difference in means, one-way analysis of variance (ANOVA) was performed with an honestly significant difference (HSD) using Tukey's test at $P\leq0.05$ (95% level of significance) using Microsoft excel for superoxide anion radical scavenging assay and DPPH assay. The antioxidant capacity and MTT assay calculation done by Microsoft Office excel 2007.

RESULTS AND DISCUSSION

The DPPH radical scavenging activity of all the fractions gradually increased in a concentration-dependent manner. The radical scavenging activities were in the order C5D>M7D>T7D>C7D>T5D>M5D. The M7D extract showed maximum percent inhibition (62.2%) at 2 mg/ml concentration and lowest in T5D (2.19%) at 0.25 mg/ml. The extracts C5D, M7D, T7D, C7D, T5D and M5D showed a percent inhibition of 62.20, 40.78, 40.45, 39.63, 21.98 and 19.67%, respectively at 2 mg/ml concentration (Table 1 and fig. 1a).

It was noticed that all the extracts were capable of scavenging hydrogen peroxide in a dose-dependent manner. The scavenging activity was in the order T7D>T5D>M7D>C5D>C7D>M5D, where T7D extract showed maximum percent inhibition of 91.30% at 2 mg/ml concentration and lowest in M5D i.e. 20.17% at 0.125 mg/ml concentration. The extracts T7D, T5D M7D, C5D, C7D and M5D showed percent inhibition of 91.30, 90.60, 89.40, 87.70, 80.90 and 78.15%, respectively at 2 mg/ml concentration (Table 1 and fig. 1b).

To investigate sprout extracts (10-100 µg/ml) for antiproliferative potential and to understand their mechanism of action, PC-3 cell line was used. To study the potential of different sprout extracts on proliferation and survival of PC-3 cells, the cells were exposed

TABLE 1: IC_{50} VALUES OF DIFFERENT EXTRACTS TREATED WITH DIFFERENT CONCENTRATION

Extracts	IC_{50}		
	MTT assay(µg/ml)	SAO assay (mg/ml)	DPPH assay (mg/ml)
M5D	111.6	0.463	5.79
M7D	81.11	0.059	2.76
C5D	95.57	0.170	1.51
C7D	71.58	0.26	2.75
T5D	63.5	0.053	5.92
T7D	43.61	0.00032	2.78

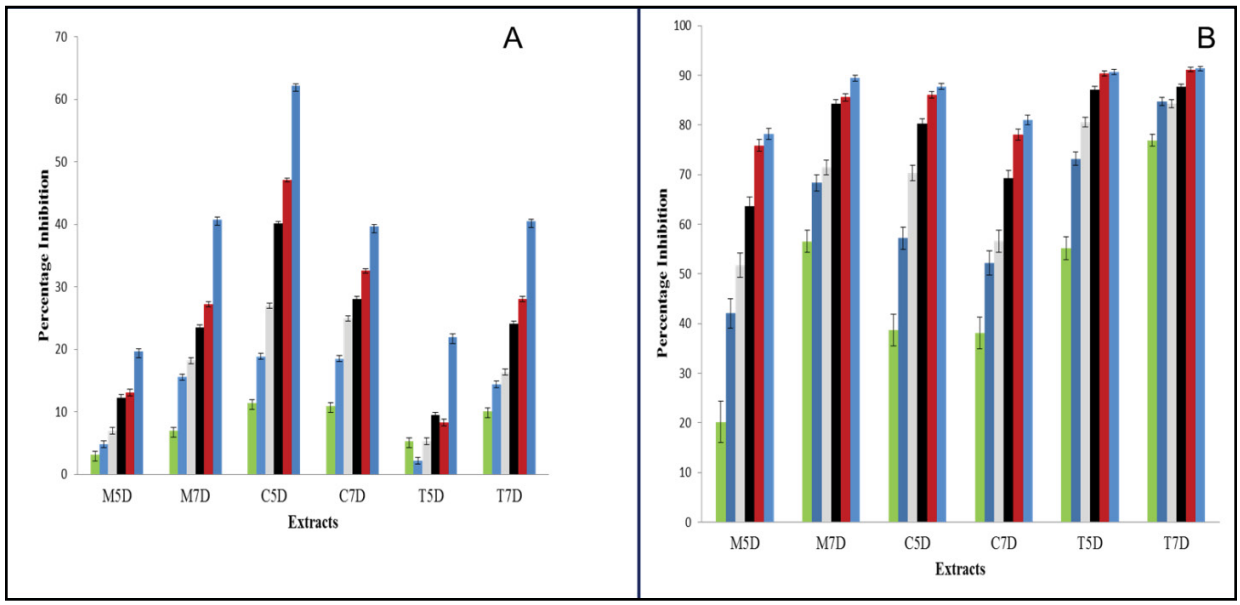

Fig. 1: DPPH and superoxide anion scavenging ability of different extracts of *Brassica*.
A. DPPH and B. Superoxide an ion scavenging ability of different extracts of *Brassica*; -■- 0.125 mg/ml, -■- 0.25 mg/ml, -■- 0.5 mg/ml, -■- 1 mg/ml, -■- 1.5 mg/ml, -■- 2 mg/ml.

to 10-100 µg/ml of extracts for 12 h. Sprout extracts induced cell death in a dose-dependent manner. The highest effective IC_{50} was reported in T7D extract i.e. 43.60 µg/ml whereas lowest effective values of 111.60 µg/ml in M5D extract. The T5D, C7D, M7D and C5D have 63.50, 71.58, 81.11 and 95.57 µg/ml IC_{50} values, respectively (Table 1). All the extracts showed a very good cytotoxic effect on the PC-3 cells therefore, they were further studied to understand the mechanism involved in cell death.

In the present study, PC-3 cells treated with plant extracts at IC_{50} value for 12 h restricted the cells in hypodiploid (sub G_0) phase of the cell cycle in concentration dependent manner as compared to the control. The sub G_0 population in M5D, T5D, C5D, M7D, T7D and C7D was 61.80, 38.30, 61.00, 47.60, 55.60 and 74.10%, respectively. The cells treated with positive control camptothecin and untreated cells have 39.40% and 15.60% cells in G_0 phase respectively and all extracts exhibited an increase in the sub G_0 population. The percentage of G_2/M phase cells was increased when cell cycle analysis was done with different extract on PC-3 cells. The cell cycle progression was significantly arrested in G_2/M phase after 12 h of treatment with different extracts (fig. 2).

ROS play an important role in depolarizing mitochondria and apoptosis induction. Flow cytometric analysis of PC-3 cells treated with different concentrations of plant extracts was done after staining with DCFH-DA, which penetrate the cells, react with cellular esterases and ROS and then metabolized into fluorescent DCF. A specific fluorescent probe for measuring hydrogen peroxides and hydroxyl radicals revealed a concentration-dependent increase in DCF positive cell population indicating generation of reactive oxygen species. The level of ROS generation was 19.00, 17.80, 16.20, 21.90, 19.70 and 26.50% in sprout extract T5D, M5D, M7D, C5D, C7D and T7D at their respective IC_{50} after 12 h exposure. The ROS generation was enhanced by 36.10% in treatment with positive control (camptothecin at its respective IC_{50}) as compared to (7.10%) untreated PC-3 cells (fig. 3).

The GC-MS analysis of an individual component in the extract was done by comparison of their mass spectra (MS) with NIST database and Adams libraries[17,18]. It was observed that 3-butenyl isothiocyanate was present in three extracts M7D, T7D and C5D. Cyclopropane, isothiocyanates present in T5D, erucin in T7D and Iberin nitrile in both T5D and C7D (Table 2). Tetradecanal and linoleic acid are the phytochemical which are present in all extracts.

A plant based diet is regarded as one of the potential chemopreventive agents. The extracts of fruits and vegetables and their bioactive components have received particular attention, and many of these have been evaluated as potential nutraceuticals and functional foods[19]. In this paper, we have focused on volatile phytochemicals in sprouts, which are present at significant levels in members of the *Brassica* family. There was also a report on comparison of bioactive

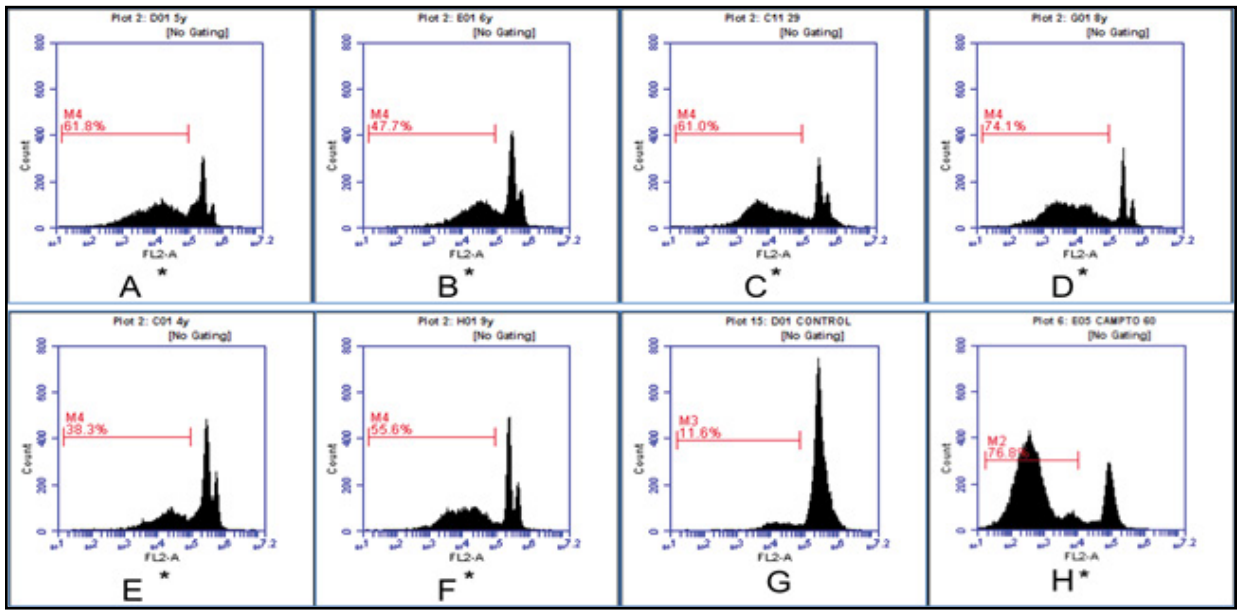

Fig. 2: The cell cycle analysis of PC-3 cells treated with sprout extract.
IC_{50} of the M5D (A), M7D (B), C5D (C), C7D (D), T5D (E) and T7D (F), respectively, compared to the untreated control cells (G) and positive control camptothecin (H).

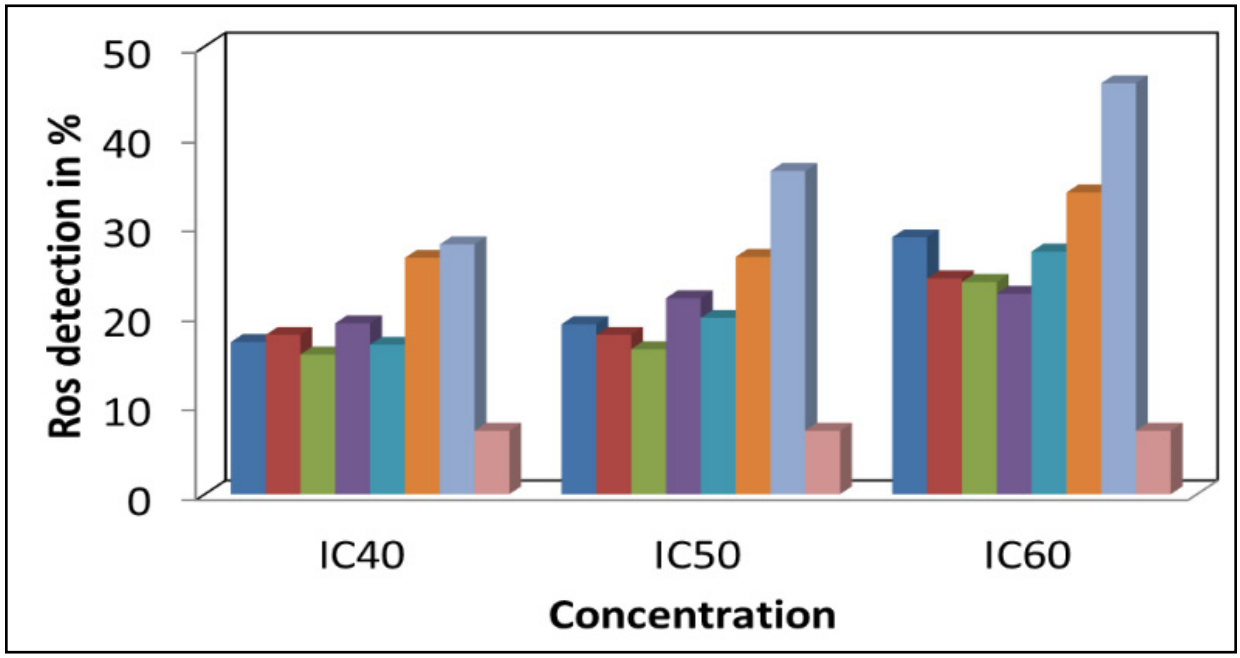

Fig. 3: Effect of plant extracts on the generation of ROS in PC-3 cells.
Effect of plant extracts on the generation of ROS in PC-3 cells at above and below IC_{50} and IC_{50} concentrations respectively, compared to untreated control cells and positive control camptothecin.; -■-T5D, -■-M5D, -■-M7D, -■-C5D, -■-C7D, -■-T7D, -■- camptothecin, -■- control.

phytochemical content and release of ITCs in selected *Brassica* sprouts[20]. Their overall protective effect has been generally attributed to the modulation of xenobiotic metabolilizing enzymes and enzymatic antioxidative defense system[21]. However, whether these effects can mainly be ascribed to a single class of molecules, e.g. glucosinolates or ITCs, or to their complex interactions with other phytochemicals present in the whole vegetable, still remains unknown. Therefore, the extracts were profiled for the presence of phytochemicals using GC-MS which showed wide variation. The cruciferous sprouts were found to be rich in many sulfur-containing compounds, such as allyl methyl sulfide and allyl propyl sulfide which are reported in the literature as strong antiproliferative agents[22]. However, till date, no study had considered

TABLE 2: DIFFERENT PHYTOCONSTITUENTS PRESENT IN EXTRACTS OF BRASSICA SPROUTS

Phytoconstituents	M7D	M5D	T7D	T5D	C7D	C5D
3-butenyl isothiocyanate	P	A	P	A	A	P
5-methylthiazole	P	A	A	A	A	A
3,5,5-trimethyl-2-cyclohexene-1-one	P	A	P	A	A	P
4,5-dimethyl-thiazole	P	P	P	A	A	A
1,3-tetradecadien-1-ol	P	A	A	A	A	A
Tridecane	P	A	A	A	A	A
1-tetradecanol	P	P	P	A	A	P
Tetradecane	P	A	P	A	A	P
Hexadecane	P	A	A	A	A	A
Tetradecan-3-yl hexanoate	P	A	A	A	A	A
2,4-di-tert-butylphenol	P	A	A	A	P	P
Octadecane	P	A	P	P	P	P
3-octadecane	P	A	A	A	A	A
N-octadecane	P	A	A	A	A	A
Oleic acid	P	A	P	P	A	A
Cyclotetradecane	P	A	A	P	P	P
Propyleneoxide	P	A	A	A	A	P
9-eicosene	P	A	A	A	A	P
Linoleic acid	P	P	P	P	P	P
Tetradecanal DB-5	P	P	P	P	P	P
Cyclohexanoneoxime	A	A	A	A	P	P
1-dodecene	A	A	A	P	A	P
Octadecyl ester	A	A	A	A	A	P
Heptadecane	A	A	A	A	A	P
1,8-Di(4-nitrophenylmethyl)-3,6-diazahomoadamantan-9-one	A	A	A	A	A	P
Eicosene	A	A	A	A	A	A
Pentacosane	A	A	A	A	A	P
Guai-1(10)-en-11-ol	A	A	A	A	A	P
Docosene	A	A	A	P	A	P
Cyclopropylisothiocyanate	A	A	A	P	A	A
Dodecyl fluoride	A	A	A	A	A	A
Iberin nitrile	A	A	A	P	P	A
Phenol, 3,5-bis(t-butyl)	A	A	A	P	A	A
1-hexadecanol	A	A	A	P	A	A
Neophytadiene	A	A	P	P	A	A
Isothiazole, 3-methyl	A	P	P	A	A	A
Erucin	A	A	P	A	A	A
1-hexadecene	A	A	P	P	A	A
(2R,4S)-2-(2-methyl-3hydroxy-5-hydroxymethylenepyridine-C4)	A	A	P	A	A	A
2H-Benzocycloheptен-2-one,3,4,4a.5,6,7,8,9-octahydro-4a-methyl	A	A	P	A	A	A
1,3,5-Triazine-2,4-diamine,6-chloro-N-ethyl	A	A	P	A	A	A
1,1,4,4-tetradeuteriobutadiene	A	A	P	A	A	A
Z,Z-8,10-Hexadecadien-1-ol	A	A	P	A	A	A
Methyl 3-oxo-5-(1-nitro-2-oxocyclododecyl)pentanoate	A	A	P	A	A	A
2,5-Furandione,3-(dodecenyl)dihydro	A	A	P	A	A	A
3,7,11,15-tetramethyl-2-hexadecen-1-ol	A	A	P	A	A	A
Cis-9,10-Epoxyoctadecanoic acid	A	A	P	A	A	A
Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis	A	A	P	A	A	A
1,2-benzenedicarboxylic acid, dibutyl ester	A	A	P	A	A	A
7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	A	A	P	A	A	A
1-nonadecene	A	A	P	A	A	A
9-tricosene, (z)-	A	A	P	A	A	A
Decanoic acid	A	A	A	A	P	A
Cyclobutane, methoxy-	A	A	A	A	P	A
Trimethylsilyl 2-[(trimethylsilyl)oxy]hexacosanoate	A	A	A	A	P	A
Phytolacteate	A	A	A	A	P	A
Decyloctylphthalate	A	A	A	A	P	A
Dodecenal	A	A	A	A	P	A

the degree of variability in antioxidant and antiproliferative potential with respect to the different developmental stage. The DPPH• radical-scavenging activity for samples of sprouts (M5D, M7D, T5D, T7D, C5D and C7D) expressed as percent inhibition from 2.19 to 62.2%. Although all the samples had considerable OH• inhibition activity whereas most of the samples statistically significantly different ($P<0.05$). In the present study, all sprouts extract showed a concentration-dependent scavenging of DPPH radical with the maximum activity of 62.2% in cauliflower (C5D). The superoxide radicals generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT. It was noticed that all the extracts exhibited scavenging of hydrogen peroxide in an amount dependent manner. Highest scavenging of hydrogen peroxide was reported by turnip seven day (T7D) i.e. 91.3% at 2 mg/ml concentration of the extract. One way ANOVA statistical analysis showed the significant effect of dose on the free radical scavenging. Our results indicated variations in phytochemicals as well as correlative changes in the antioxidant capacity of different sprouts extracts.

Chemotherapeutic drugs are known to induce cytotoxicity in tumor cells through diverse mechanisms, in which signaling events play an important role depending upon the cell type and stimulus[23,24]. In the current study, antiproliferative activity was detected by the MTT assay, a colorimetric method for determining the number of viable cells in proliferation. The crude sprouts extract (10-100 µg/ml) showed high antiproliferative activity in the human prostate cancer (PC-3) cells (Table 1). A significant dose-dependent antiproliferative effect was observed in human prostate cancer cells (PC-3) in the presence of different sprouts extract in the present findings. The highest antiproliferative activity was found with the turnip seven-day sprout (T7D) with IC_{50} of 43.6 µg/ml and lowest effective values of 111.6 µg/ml in mustard five-day sprout (M5D) extract. The literature studies revealed the lowest effective dose for HT-29 colon carcinoma cells was 0.25 g eq ww/ml for broccoli and 0.50 g eq ww/ml for cauliflower and brussels sprouts[25].

As all the extracts showed a significant effect on the PC-3 cell line, therefore they were further studies to know the mechanism involved in cell death. Furthermore, the cytostatic effect of the individual biologically active compound is less than the crude plant sprouts extracts[26]. Results of MTT assay and cell morphological changes are supported further by analysis the cell cycle and intracellular measurement of ROS. There are reports which show that sulforaphane exerts its anti-proliferative effect by arresting the cell cycle; this arrest has been documented in the colon, prostate, breast, bladder and T cells[27]. It is well known that cell growth and proliferation of mammalian cells are dependent on cell cycle progression[28]. The fluorescence intensity of sub G_0 cell fraction represented the apoptotic cell population. Previous studies revealed that anticancer agents arrest the cell cycle at the G_0/G_1, S or G_2/M phase and then induce apoptotic cell death[15,24,29]. The cell cycle arrest has become an appreciated target for management and treatment of tumor cells with cytotoxic agents. In the present study, apoptotic cell death increased significantly after treatment with extracts for 12 h, as a gradual increase in sub G_0 population and arrest of PC-3 cells in sub G_2M phase. It has also been mentioned in the literature that benzyl isothiocyanate induces arrest of cells at G_2/M phase and apoptosis in human melanoma A375.S2 cells through ROS[30]. Cancer cells undergo apoptosis either by a generation of free radicals or by depletion of endogenous antioxidants[31]. In the present study, an elevated level of intracellular ROS was observed in the PC-3 cells with an increase in the concentration of sprouts extracts. This clearly indicates that sprout extracts induced cytotoxicity by a generation of ROS. Likewise, the studies in the literature also revealed ROS mediated apoptosis by pomegranate peel extract and in sprouts of crucifers[32,33]. Chemoprevention, a relatively new strategy to prevent cancer, depends on the use of nontoxic chemical substances, to block, reverse or retard the process of carcinogenesis. DNA damage activates the G_2/M checkpoint, which prevents damaged cells in G_2 phase from entering into mitosis. It is pertinent to mention that the present study report first time that the extraction of sprouts with DCM *in vitro* cytotoxicity against PC-3 cell lines and induce apoptosis as evidenced by a measurement of several biological endpoints of apoptosis such as the appearance of apoptotic bodies and increase in sub G_0 DNA fraction in cells. Although, the extracts of different sprouts exhibited one or the other bioactivity but the C5D extract consistently showed good activity in most of the assays.

In conclusion, this study has shown that plant derived sprout byproducts are a good and cheap source of antioxidants which could be industrially exploited. The extracts showed the highest scavenging activities

against superoxide radical followed by and DPPH. Further, *in vitro* antiproliferative study supports that sprouts are a good source of anticancer agents and the activity can be correlated to phytochemicals high amount of sulfur rich compounds funding.

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Conflict of interest:

There are no conflicts of interest.

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