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Research Article

EVALUATION ON LIPID PEROXIDATION AND ANTIPROLIFERATIVE ACTIVITIES OF *RAPHANUS SATIVUS* L.

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ABSTRACT

Raphanus sativus L. is a most commonly used vegetable belonging to the family Cruciferae. In the present study, the plant material was procured and processed for cold extraction procedure using 70% ethanol. The extract was screened for the qualitative phytochemical analysis, in vitro lipid peroxidation inhibition and antiproliferative activites. From the results, *Raphanus sativus* L. revealed potent lipid peroxidation inhibition with an IC50 value 281.9 µg/mL compared to standard ascorbic acid and BHT. Further, MTT assay was used to measure cell proliferation against NIH-3T3, DLA and MCF7 cell lines and the significant results were recorded in the plant extract with an IC50 value of 415.4 µg/mL, 423.2 µg/mL and 703.5 µg/mL respectively.
Keywords: Phytochemicals, anti-proliferative, lipid peroxidation, MTT, Cell lines, *Raphanus sativus*.

INTRODUCTION

Free radicals now appear the fundamental mechanism underlying a number of human neurologic and other disorders. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes and play a role in the long-term complication of many degenerative diseases. Antioxidants are free radical scavengers and hence prevent and repair damage done by these free radicals (Polterait, 1997; Prior, 2003; Saha et al. 2008)

Cancer is a group of diseases characterized by the over proliferation of the cells. Reports in literature indicate that about 30-40% types of cancers are directly or indirectly linked to improper diet and related factors (Czapski, 2009). Epidemiological studies indicated optimistic association between intake of fruits and vegetables and reduced mortality from cancers, heart and other degenerative diseases (Kaur and Kapoor, 2001; Art and Hollman, 2005; Scalbert et al. 2005). One group of vegetables that has been widely approved for its beneficial effects on human health is that of the Cruciferae vegetables. *Raphanus sativus* L. is one of the most commonly used vegetables in India. It is

a fast-growing, annual, cool-season crop. It is a very good source of antioxidants, minerals, vitamins and dietary fiber. In India, the plant has several folkloric uses as purgative, stimulant, antiscorbutic, diuretic and anticancer properties (Pandey, 2012). Therefore the present investigation was undertaken to evaluate the lipid peroxidation inhibition and antiproliferative activities of *Raphanus sativus* L.

MATERIALS AND METHODS

Collection of *Raphanus sativus* L

Raphanus sativus L (Radish) of tropical India was procured from a local market of Shimoga, Karnataka and was authenticated Mr. Avinash K, Assistant Horticulture Officer, Kadur, Chikmagalur District, Karnataka, India. The collected material was processed for extraction procedures.

Extraction

After selection, vegetable was washed under running tap water followed by washing with distilled water to remove the surface debris. The leaf portion and the outer most root skin layer of *Raphanus sativus* were removed and the remaining edible root portion was used for the analysis. Exactly 1Kg of vegetable was weighed. Finally, the above prepared vegetable sample was chopped into small pieces using cutter and later minced using a mixer grinder and finely macerated. After homogenization, it was extracted in 70% ethanol for 7 days in dark in the room temperature with intermittent shaking. After 7 days, the whole extract was filtered using muslin cloth at first and then through Whatman No. 1 filter paper and the filtrate was concentrated using rotary evaporator. The yield of crude extract obtained were noted, stored in desiccators for maximum of 3 days; later preserved in deep freezer (-20^{0}C) for further use (Jamuna et al. 2015).

Yield calculation

The yield of the extract was calculated by using the following equation:

Percentage of extraction yield = m1/m0 × 100

Where m1 is mass of the extract in gram and m0 is mass of sample in gram.

Qualitative Phytochemical Analysis

The preliminary qualitative phytochemical studies were performed for testing the different chemical groups present in ethanolic extracts of *Raphanus sativus* L. (Trease and Evans, 1978; Kokate et al. 1990; and Khandelwal, 2006).

Lipid peroxidation (LPO) inhibition assay

TBA reacts with malondialdehyde (MDA) to form a diadduct, a pink chromogen, which can be detected according to the method of Halliwell and Guttridge (1989). 10% of mice liver (CPCSEA Reg. No. 238/2014) homogenate in 0.15 M potassium chloride was prepared. 0.5 ml of liver homogenate and 1 ml of *Raphanus sativus* extract at different concentrations were taken in test tubes. Lipid peroxidation was induced by adding ferrous sulfate (50 µl, 0.07 M) and incubated at room temperature for 30 min. The reaction was stopped by adding chilled acetic acid (1.5 ml, 20%, pH 3.5) containing 20% TCA followed by the addition of TBA (50 µl, 0.8% TBA in 1.1% SDS). The content was mixed thoroughly and incubated in boiling water bath for 60 min. After cooling, 5 ml of butanol was added and centrifuged at 3000 rpm for 10 min. Absorbance of the organic supernatant was measured at 532 nm. Percentage of inhibition was calculated using the following formula:

$$\% \text{ inhibition} = [(\text{control} - \text{test})/\text{control}] \times 100$$

where control is the absorbance of the control reaction and test is the absorbance of the extract reaction.

The results were expressed as IC_{50}. All the tests were performed in triplicate and for IC_{50} values the graph was plotted with the average of the three determinations.

In vitro antiproliferative activity

Cytotoxicity of *Raphanus sativus* L. was measured by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay.

Cell culture

For the present investigation three different cell lines of varying origin were selected. Dalton's lymphoma (DL) cells of mouse origin; Breast cancer MCF-7 and NIH-3T3 cell lines (NCCS, Pune, India) of human origin. The cells were cultured in Dolbecco's modified Eagle's medium (DMEM) medium supplied with 10% heat-inactivated FBS (Invitrogen, USA) and 1% Penicillin-Streptomycin (Sigma-aldrich, USA) and 0.37% sodium bi carbonate (Sigma-aldrich, USA) was used at 37°C in a 5% CO2 enriched humidified incubator (Thermo scientific, USA) with 98% humidity.

Determination of Antiproliferative Activity by MTT assay

The antiproliferative activity of the extracts against the human cancer cell lines was tested using the microtitration colorimetric method of MTT reduction (Mosmann, 1983) with

minor modification. MTT is used to determine cell viability in cell proliferation and cytotoxicity assays. Exponential-phase cells that were 80%–90% confluent were harvested from maintenance cultures and counted using a hemocytometer with trypan blue solution. Cell suspensions (100 µL) were dispensed in triplicate in 96-well culture plates at optimized concentrations of ~1.0 × 106 cells/mL per cancer cell line. After 24-h incubation at 37°C, 100 µL culture medium was removed from the wells and 100 µL fresh medium containing the extracts (50, 100, 200, 400, 800 µg/mL) was added to each well and incubated for another 48 h. Wells containing DMEM or RPMI 1640 were used as the negative controls. At the end of the treatment period, the medium in each well was aspirated and replaced with 20 µL of 5 mg MTT working solution (MTT stock solution mixed with medium to attain a final concentration of 0.5 mg/mL). Briefly, MTT powder was dissolved in PBS to form an MTT stock solution (5 mg/mL). The stock solution was filter-sterilized through a 0.22 µm filter and stored at −20°C until used. The cells were incubated at 37°C for 4 h, and then the medium was aspirated and replaced with 100 µL DMSO to dissolve the formazan crystals formed. The cells were incubated for 10 min and the absorbance (OD) of each well was read using micro-ELISA reader (Robonics, India) at 570nm. The results were produced from three independent experiments, and each experiment was performed in triplicate for each cell line. The cytotoxicity was obtained by comparing the absorbance between the samples and the control blank. The values were then used to calculate the concentration of plant extract required to cause a 50% reduction (IC50) in growth (cell number) for each cell lines. The % of cell inhibition was determined using following formula:

$$\% \text{ inhibition} = [(A_{control} - A_{test})/A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance of the extract reaction.

Statistical Analysis

The experimental data were reported as mean ± SEM of three parallel measurements. One way analysis of variance was performed by ANOVA procedures and the results were correlated.

RESULTS

Extraction: The extraction of bioactive compounds from plant materials is the first step in the utilization of phytochemicals in the preparation of dietary supplements or nutraceuticals, food ingredients, pharmaceutical products. The result revealed that Radish showed the percentage yield of 49.85g per Kg of plant material.

Qualitative Phytochemical Analysis

The results of qualitative phytochemical analysis revealed that the *Raphanus sativus* L extract showed the presence for several bioactive compounds viz. polyphenols, flavonoids, terpenoids, steroids, glycosides, alkaloids and saponins (Table 1).

Table 1: Qualitative phytochemical analysis of *Raphanus sativus* L vegetable extract

Tests	Radish
Steroids	+
Glycosides	+
Terpenoids	+
Saponins	+
Alkaloids	+
Flavonoids	+
Tannins and Polyphenols	+

Lipid peroxidation (LPO) inhibition assay

In the present study, the extract of *Raphanus sativus* L. was investigated in comparison with the known antioxidants ascorbic acid and BHT. The IC50 values for inhibition of lipid peroxidation activity was found to be 281.9 µg/mL, whereas standard ascorbic acid and BHT recorded IC50 values of 29.8 and 81.2 µg/mL respectively.

Determination of Antiproliferative Activity by MTT assay

The results of the antiproliferative activity of *Raphanus sativus* L. at different concentrations (50, 100, 200, 400 and 800 µg/mL) against NIH-3T3, DLA and MCF7 is depicted in Fig. 1. The cell proliferation inhibition was registered in a dose dependent manner with an IC50 value of 415.4 µg/mL, 423.2 µg/mL and 703.5 µg/mL for three different cell lines – NIH-3T3, DLA and MCF7respectively.

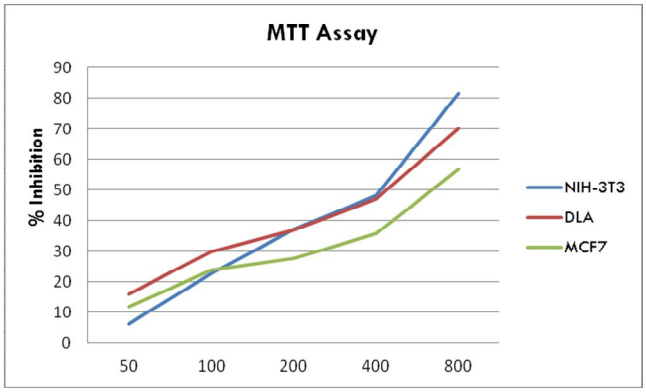

Fig 1. Antiproliferative activity of *Raphanus sativus* L. against NIH-3T3, DLA and MCF7 cell lines

DISCUSSION

Natural resources are an indispensable source of bioactive compounds. A large number of drugs have been developed in medicinal practice from natural products (Amador et al. 2003). In the present investigation *Raphanus sativus* L. extract recorded the presence for several bioactive compounds viz. polyphenols, flavonoids, terpenoids, steroids, glycosides, alkaloids and saponins. Therefore the study encourages for lipid peroxidation inhibition and antiproliferative activities.

Lipid peroxidation as a consequence of free radical activity in food and lipids is believed to proceeds through radical mediated abstraction of hydrogen atoms in polyunsaturated fatty acid (PUFA) (Li et al. 2008).

Oxidation of lipids, which is the main cause of quality deterioration in many food systems, may lead to off-flavors and formation of toxic compounds, and may lower the quality and nutritional value of foods. Furthermore, lipid oxidation is also associated with aging, membrane damage, heart disease and cancer (Ramarathnam et al. 1995). Therefore, a growing interest in the identification of an antioxidant compound from food materials has been developed. Unsaturated lipids in liver tissue are very susceptible to peroxidation when they are exposed to reactive oxygen species (ROS). In the present investigation the liver tissue is incubated in presence of ROS generating system FeSO4 and examined the effect on the tissue homogenates by measuring the optical density (OD) at 532nm. The results revealed that *Raphanus sativus* L. had lipid peroxidation inhibition activity which is compared to standards, ascorbic acid and BHT.

Cancer appears to be a major cause of morbidity and mortality and runs in the top three causes of death worldwide, especially in the developed countries. Conventional treatments such as surgical removal and chemotherapy have not yet improved the survival rate over the past two decades (Kim et al. 2013). Therefore, there is a growing interest on natural and safer antioxidants. It is determined that almost 60% of anticancer drugs are of natural origin (Gulcin, 2006; Grever, 2001).

In the present study, the cytotoxicity of *Raphanus sativus* L. on NIH-3T3, DLA and MCF7 cells were determined by the MTT assay. The method is far superior to the previously mentioned methods because it is easy to use, safe, has a high reproducibility, and is widely used in both cell viability and cytotoxicity tests. The 3T3 cell line is the standard fibroblast immortal cell line originally obtained from Swiss albino mouse embryo tissue (George Todaro and Howard Green, 1963). MCF-7 breast cancer cells serve as an excellent in vitro model for studying the mechanism of tumor response as well as complex relationships between binding and biological actions of hormones. (Dillon et al. 2010). DLA cell line is a lymphoma caused in the murine lymphatic system. Hence, this tumour cell line is reliable model for screening the potent cytotoxic molecule (Nagy et al. 1995). Our observations on cytotoxicity of *Raphanus sativus* L. extract showed significant in vitro antiproliferative activity.

The qualitative analysis of the plant, *Raphanus sativus* L. showed protective phytochemicals including phenols, flavonoids etc. Furthermore, epidemiological studies have shown that a diet rich in cruciferous vegetables can lower the risk of various cancers (Yuan et al. 1998). The major active compounds in cruciferous vegetables, the derivatives of glucosinolates viz. indole-3-carbinol, sulforaphane, isothiocyanates etc. exhibit promising cancer protective properties (Cover et al. 1998). The significant antiproliferative activity as well as inhibition of lipid peroxidation might be due to the presence of these phytochemicals.

CONCLUSION

On the basis of the results obtained in the present study, it is concluded that *Raphanus sativus* L. exhibited significant in vitro lipid peroxide inhibition and antiproliferative activities. The antiproliferative activity could be attributed to

augmentory lipid peroxide inhibition due to the presence of its phytochemicals and further detailed studies are underway.

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