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Original Research Article

Exploring *in vitro* cytotoxicity of some seed spices against human cancer cell lines

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Abstract

In the present research work, *in vitro* anticancer potential of methanolic extract of some seed spices was evaluated *via* SRB assay. The anti-cancer activity was determined by the cytotoxic potential of test material at 100 µg/ml. Cells were allowed to grow for 24 h on 96-well flat bottom tissue culture plates and cells were further allowed to grow in the presence of test material for 48 h. Cell growth was terminated by addition of 50% (w/v) tricarboxylic acid and cells were stained with SRB dye. Excess dye was removed by washing with 1% (v/v) acetic acid and bound dye was dissolved in Tris buffer.OD was taken at 540 nm. *Cuminum cyminum* displayed *in vitro* cytotoxic effect against CNS human cancer cell line and *Piper nigrum* showed *in vitro* cytotoxic effect against human cancer cell line from colon origin. Further, isolation and characterization of active ingredients is required for the development of anticancer drugs.

Keywords: *Cuminum cyminum*, *Piper nigrum*, SRB assay, *In vitro* cytotoxicity

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1. Introduction

Coriandrum sativum commonly known as coriander or dhaniya, is cultivated all over the world for its use, not only in the indigenous medicines, but also as one of the ingredients of all spicy foods especially of Asian countries. Various parts of this plant such as seed, leaves, flower and fruit possess diuretic, antioxidant, ant-diabetic, anti-convulsant, sedative hypnotic, anti-microbial, antimutagenic and anthelmintic activity.1 Both aqueous and ethanolic seed extracts of *C. sativum* exhibited *in vitro* cytotoxicity on MCF-7 human cancer cell line. The inhibition of cancer cell growth was highest at the concentration of 500 µg/mL and the IC_{50} values estimated were found better in the ethanolic extract than in aqueous.2 *Cuminum cyminum* commonly known as cumin / jeera is one of the old cultivated medicinal food herbs in Asia, Africa and Europe. Its seeds have been commonly used for culinary, flavoring purposes and folklore therapy since antiquity in various countries. Four flavone structures namely uteolin, apigenin, luteolin-7-O-glucoside, apigenin-7-O-glucoside and cuminoid A were purified and identified from ethyl acetate and hexane fractions respectively and cytotoxicity analysis of pure compounds against breast cancer cell lines (MCF-7 and MDA-MB-231) and normal cell line (NIH/3T3) by MTT assay revealed that luteolin-7-O-glucoside showed potent inhibitory activity against MCF-7 cell line (IC_{50} of 3.98 mg/ml) with selectivity index of 8.0 concluding that significant role was played by flavonoids especially luteolin-7-O glucoside in cytotoxic effect of *C. cyminum* fruits.3 *Foeniculum vulgare* commonly known as fennel or saunf, is used in traditional medicine for a wide range of ailments related to digestive, endocrine, reproductive and respiratory systems. It has several *in vitro* and *in vivo* pharmacological properties such as antimicrobial, antiviral, anti-inflammatory, antimutagenic, antinociceptive, antipyretic, antispasmodic, antithrombotic, apoptotic, cardiovascular, chemomodulatory, antitumor, hepatoprotective, hypoglycemic, hypolipidemic and memory enhancing property.4 The ethanolic extract of *F. vulgare* was found to exhibit the most significant anticancer activity against Hela cells with IC_{50} value of 19.97 µg/mL.5 *Piper nigrum* or black pepper, also known as kali mirch, is commonly used as a spice and is considered as the “King of spices”. It is used in different systems of medicine like

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Ayurvedic / Unani System of Medicine for its medicinal properties which include antibacterial, antifungal, antiapoptotic, antidepressant, antidiarrheal, antiinflammatory, antimutagenic, antioxidative, antipyretic, antispasmodic, antitumor, anticold, anticough, anticolic and antidysentery.6 Piperine, a major alkaloid constituent of black pepper, showed cytotoxicity against prostate cancer cells *viz*., LNCaP, 22RV1, PC-3 and DU-145 in a dose dependent manner.7 *Trigonella foenum-graecum* / fenugreek, commonly known as methi is cultivated worldwide as a semiarid crop and shows antidiabetic, hypoglycemic, antiallergic and lactation induction properties.8 The aqueous extract of fenugreek seeds showed *in vitro* cytotoxic effect at higher concentrations, whereas at lower concentrations it increased cell viability, suggesting the induction of dose dependent hormetic response in human breast cancer cell lines (T-47D and ZR-75-1). The alcoholic fenugreek extract was markedly cytotoxic to both the breast cancer cell lines, where multiple cell division defects together with apoptosis were apparent in the affected cells.9

2. Material and Methods

2.1. Authentication and collection

The above mentioned seed spices were collected from National Research Centre on Seed Spices (NRCSS), Ajmer, Rajasthan.

2.2. Crushing

The seeds were crushed and the coarse plant material was then extracted with methanol at room temperature (35 ^{0}C) for bioevaluation.

2.3. Extraction method

Powdered dried seed material was placed in a percolator of appropriate size. The material was then submerged in 99% (v/v) methanol depending on the need. Standard protocol[10] was followed for the extraction. Dried plant material (100 g) was placed in a conical glass percolator. Sufficient quantity of solvent was added so as to submerge the plant material. After standing for about 16 h (overnight), the percolate was collected and filtered if required. The process was repeated four times, which was generally sufficient for exhaustive extraction of the plant material. The methanolic extract (collected in four attempts) was evaporated to dryness under reduced pressure at 60 ^{0}C using rotavapor and round bottom flask (RBF).The final drying was done in a vacuum desiccator. The dried extract was scrapped off from the RBF and transferred to a tared wide mouth glass container of appropriate size. The container was weighed to calculate the quantity of the extract obtained. This formed the "stock extract" and generally, 8 to 10 g crude extract was obtained from 100 g of the dried plant material. The extracts obtained, were stored at –20 ^{0}C under desiccation in deep freezer for further testing.

2.4. Stock solution & Working test solution (200 µg/ml)

A stock solution of 20 mg/ml was prepared in DMSO. For 99% (v/v) methanolic extract, DMSO was used. Stock solutions were prepared atleast one day in advance. Stock solutions were not filtered / sterilized, but microbial contamination was controlled by the addition of gentamycin in complete growth medium used for dilution of stock solutions to prepare working test solutions. On the day of assay, an aliquot of frozen stock solution was thawed at room temperature. Working test solution was prepared by dilution of stock solution with gentamycin medium. (10 µl of stock solution +990 µl of gentamycin medium = 1000 µl).

Figure 1: *Coriandrum sativum* (Coriander)

Figure 2: *Cuminum cyminum* (Cumin)

Figure 3: *Foeniculum vulgare* (Fennel)

Figure 4: *Piper nigrum* **(**Black pepper)

Figure 5: *Trigonella foenum – graecum* (Fenugreek

Table 1: Growth inhibitory effect of methanolic extracts of seed spices along with positive controls against human cancer cell lines

Plant	Plant part used	Conc.(µg/ml)	Human cancer cell lines from seven different tissues						
			Breast	CNS	Colon	Liver	Lung	Pancreatic	Prostate
			MCF-7	SHSY-5Y	HCT-116	HEP-2	A-549	MIAPACA	PC-3
			Growth Inhibition (%)						
Coriandrum sativum	Seeds	100	17	35	00	01	01	02	00
Cuminum cyminum	Seeds	100	17	100	21	31	30	11	52
Foeniculum vulgare	Seeds	100	52	31	00	12	01	12	00
Piper nigrum	Seeds	100	45	52	86	19	64	23	56
Trigonella foenum-graecum	Seeds	100	12	52	00	02	09	03	00
Positive controls (standard drugs)		Conc.(µM)							
Doxorubicin		1	65	-	-	-	-	-	-
Mitomycin-C		1	-	-	-	78	-	-	66
Paclitaxel		1	-	60	-	-	78	65	-
5-Fluorouracil		20	-	-	52	-	-	-	-

2.5. Positive controls

Positive controls were initially prepared with DW (doxorubicin, mitomycin-C,) and DMSO (paclitaxel) and were further prepared in gentamycin medium to obtain working test solutions.

2.6. Determination of cytotoxicity

Cytotoxicity was performed against various human cancer cell lines from different tissues.[11] Number of 96-well flat bottom tissue culture plates was dependent upon the number of test samples along with appropriate positive controls. There were four types of wells in the tissue culture plates, control blank (CB, without cells, complete growth medium only) and control growth (GC, with cells alone in the absence of test material) to determine 100% growth. The growth in the presence of test material was determined from the difference of test growth (GT, cells with test material) and test control (CT, test material without cells). Systematic bioassays were performed on different human cancer cell lines *via* SRB process[12] using SRB dye. The SRB assay is simpler, faster and more sensitive. It provides better linearity with cell number and was less sensitive to environmental fluctuations.

2.7. Calculations

The cell growth was determined by subtracting average absorbance value of respective blank from the average absorbance value of experimental set. Percent growth in presence of test material was calculated as under:

1. OD Change in Presence of Control = Mean OD of Control – Mean OD of Blank
2. OD Change in Presence of Test Sample = Mean OD of Test sample – Mean OD of Blank
3. % Growth in Presence of Control = 100/OD change in presence of control

4. % Growth in Presence of Test Sample = (% growth in presence of control) × OD change in presence of test sample
5. % Inhibition by Test Sample = 100 – % growth in presence of test sample

2.8 Criteria for activity

The growth inhibition of 70% or above was considered active while testing extracts.

3. Results and Discussion

The results are summarized in **Table 1** that demonstrated that the methanolic seed extract from *Coriandrum sativum* did not exhibit *in vitro* cytotoxicity against any of the human cancer cell lines used in the present study. The growth inhibition by this extract was observed in the range of 00-35%, which is not considered significant. The methanolic extract derived from the seeds of *Cuminum cyminum* showed significant *in vitro* cytotoxic effect against one human cancer cell line *i.e.*, SHSY-5Y from CNS origin as maximum growth inhibition *i.e.,* 100% was observed in this case. The extract also showed 52% growth inhibition against prostate cancer cells (PC-3), 31% growth inhibition against liver cancer cells (HEP-2), 30% growth inhibition against lung cancer cells (A-549), 21% growth inhibition against colon cancer cells (HCT-116), 17% growth inhibition against MCF-7 (breast cancer cell line) and 11% growth inhibition against MIAPACA (pancreatic cancer cell line). The observations demonstrated that the methanolic extract derived from the seeds of *Foeniculum vulgare* suppressed the proliferation of breast cancer cells (MCF-7) by 52% and CNS (SHSY-5Y) cancer cells by 31%, however in case of other cancer cells, the growth inhibition was found in the range of 0-12%.The methanolic extract derived from the seed part of *Piper nigrum* showed significant *in vitro* cytotoxic effect against one human cancer cell line *i.e.,* HCT-116 from colon origin as maximum growth inhibition *i.e.,* 86% was observed in this case. The extract also showed 64 % growth inhibition against lung cancer cells (A-549), 56% growth inhibition against prostate cancer cells (PC-3), 52 % growth inhibition against CNS (SHSY-5Y) cancer cells,45 % growth inhibition against MCF-7 (breast cancer cell line), 23% growth inhibition against MIAPACA (pancreatic cancer cell line) and 19 % growth inhibition against liver cancer cells (HEP-2).The methanolic extract from the leaves of *Trigonella foenum – graecum* showed 52 % growth inhibition against CNS (SHSY-5Y) cancer cells. In case of other cancer cells (MCF-7, HCT-116, HEP-2, A-549, MIAPACA, PC-3) from breast, colon, liver, lung, pancreatic, prostate origin, the growth inhibition was observed in the range of 00-12%.

Cancer, now a days, is becoming a big load on families and economies. Presently, more than hundred types of cancer are known, the most commonly occurring ones are breast, colon, cervical, liver, lung, oral, ovary and prostate cancer. In recent years, cancer research has become a major area of scientific research supporting the foundations of modern biology to a great extent. Diverse biological disciplines such as cytogenetics, virology, cell biology, molecular genetics, epidemiology and biochemistry together with the clinical sciences have close links in their research of how cancer develops and to find remedies to stop the abnormal growth that is characteristic of cancerous cells. Despite the recent advances in surgery, endocrine therapy, radiotherapy and chemotherapy, it is considered that the management of cancer is still not up to the mark and we are in emergent need of drugs for the treatment of cancer having no side effects. On the other hand, there is a continuous / urgent need to discover new anticancer compounds with diverse chemical structures and novel mechanism of action due to an alarming increase in the cancer cases all over the world. Therefore, screening of plant extracts has been of great interest to scientists and plants extracts / phytochemicals with known anticancer properties can be of great importance in therapeutic treatments. Keeping this in mind, the present investigation was carried out to evaluate the *in vitro* anticancer potential of some seed spices against seven human cancer cell lines from seven different tissues *via* methanolic extract. *In vitro* assay for cytotoxic activity was conducted by using SRB dye with appropriate positive controls and the results revealed that *Cuminum cyminum* (cumin) and *Piper nigrum* (black pepper) showed (cell line specific) *in vitro* cytotoxic activity with one or the other human cancer cell line. Cumin exhibited maximum *in vitro* cytotoxic effect against one human cancer cell line *i.e.,* SHSY-5Y from CNS origin with 100% growth inhibition. Black pepper produced maximum *in vitro* cytotoxicity against one human cancer cell line *i.e.,* HCT-116 from colon origin with 86% growth inhibition.

4. Conclusion

Several spices have exerted anticancer effects including lung, liver, breast, stomach, colorectum, cervix, and prostate cancers. Direct extract, essential oil and compounds isolated from spices are commonly studied. Some spices' compounds exert anticancer properties in both cells and animal models, suggesting they might be effective in human cancer. Several components of spices show their anticancer effects in the digestive system, indicating these spices might be a healthy dietary means to prevent cancer directly. Therefore, focus has now shifted towards natural products such as fruits, vegetables, spices and other parts of plants to save the future cancer treatment. The results from present investigation forms a good basis for selection of these seed spices (cumin, black pepper) for further phytochemical and pharmacological analysis. The results produced in the research support folkloric usage of the studied seed spices and showed that the extracts obtained from the seed part of the of the spices possess certain cytotoxic constituents that can be used for developing anticancer agents for cancer therapy. The active extracts can be subjected to the isolation of active molecules.

5. Source of Funding

None.

6. Conflict of Interest

None.

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