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Original Research Article

Kalonji and carom seeds possess *in vitro* anticancer efficacy

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Abstract

In the present research work, *in vitro* anticancer potential of methanolic extract of kalonji and carom seeds was evaluated *via* SRB process. 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 and growth inhibition of 70% or above was considered active for our bioassay purpose. Kalonji displayed *in vitro* cytotoxic effect against two human cancer cell lines from two different tissues and *Trachyspermum ammi* showed *in vitro* cytotoxic effect against four human cancer cell lines originated from four different tissues.

Keywords: *Nigella sativa*, *Trachyspermum ammi*, SRB process, *in vitro* anticancer

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1. Introduction

Nigella sativa commonly known as black cumin or kalonji is an annual and seasonal (winter) cross pollinated crop belonging to the family Ranunculaceae. It is native to Southern Europe, North Africa and Southwest Asia and is also cultivated in different parts of India such as Rajasthan, Punjab, Himachal Pradesh, Madhya Pradesh, Bihar, Bengal, Assam and Maharashtra. It is used as an important drug in the folk medicine in the Middle East and some Asian countries for its biological activities and therapeutic potential. It shows diuretic, antidiabetic, analgesic, antimicrobial, antihypertensive, anticancer, anti-inflammatory, spasmolytic, gastroprotective, immunomodulatory and antioxidant properties.[1] Nigella seed oil showed inhibition of cell viability by 50% in AGS human stomach cancer cells with IC_{50} value of 3.1 mg/mL and methanolic extract exerted significant cytotoxicity in AGS cells in a concentration dependent manner, with IC_{50} value 0.48 mg/mL (24 hours incubation). The results also revealed that octahydropyrazino [2,1-a:5,4-a'] diisoquinoline derivative (OM-90) (IC_{50} = 24 ± 2 μM) was more cytotoxic as compared to etoposide (IC_{50} = 5 ± 2 μM).[2] *Trachyspermum ammi* commonly calledcarom seeds / ajwain is a native of Egypt belonging to the family Apiaceae. The fruit possesses stimulant, antispasmodic and carminative properties and traditionally it is used as an important remedial agent for flatulence, diarrhoea, abdominal tumors, abdominal pains, piles, lack of appetite, galactogogue, asthma and amenorrhoea. It also possesses antifungal, antioxidant, antimicrobial, antinociceptive, cytotoxic, hypolipidemic, antihypertensive, antispasmodic, diuretic, nematicidal, anthelmintic and antifilarial activities.[3] Ajwain seed essential oil (TSEO) and poly lactideco-glycolide (PLGA) based nanoparticles showed significant cytotoxic effect on HT-29 cells in a dose and time-dependent manner. The TSEO-PNP showed three main anticancer activities on HT-29 colon cancer cells including oxidant reduction, apoptosis induction and angiogenesis suppression.[4] In the present study, we have evaluated *in vitro* cytotoxic effect of kalonji and carom seeds against various human cancer cell lines.

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2. Materials and Methods

2.1. Authentication and collection

The above mentioned kalonji and carom seeds 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 bio evaluation.

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[5] 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).

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.[6] 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[7] 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.

3. 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.

Table 1: Growth inhibitory effect of *Nigella sativa* along with positive controls against human cancer cell lines

Plant part used	**Extract**	**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 (%)						
Seeds	Methanolic	100	97	32	31	80	51	14	42
Positive controls (standard drugs)		Conc.(µM)							
Doxorubicin		1	65	-	-	-	-	-	-
Mitomycin-C		1	-	-	-	78	-	-	66
Paclitaxel		1	-	60	-	-	78	65	-
5-Fluorouracil		20	-	-	52	-	-	-	-

Growth inhibition of 70% or above has been indicated in bold number.
The mark (-) indicates that the particular human cancer cell line was not treated with that particular positive control

Table 2: Growth inhibitory effect of *Trachyspermum ammi* along with positive controls against human cancer cell lines

Plant part used	**Extract**	**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 (%)						
Seeds	Methanolic	100	80	21	70	09	90	31	100
Positive controls (standard drugs)		Conc.(µM)							
Doxorubicin		1	65	-	-	-	-	-	-
Mitomycin-C		1	-	-	-	78	-	-	66
Paclitaxel		1	-	60	-	-	78	65	-
5-Fluorouracil		20	-	-	52	-	-	-	-

Growth inhibition of 70% or above has been indicated in bold numbers. The mark (-) indicates that the particular human cancer cell line was not treated with that particular positive control.

4. Results and Discussion

The results are summarized in **Table 1** regarding the *in vitro* anticancer potential of methanolic extract from the seed part of *Nigella sativa*, which showed the significant activity of extract against two human cancer cell lines from breast and liver origin. The growth inhibition of 97% and 80% was produced by this extract against MCF-7 and HEP-2 respectively.

The range of growth inhibition produced by the extract against other five human cancer cell lines was 14-51%, which is considered inactive.

The observations produced by the methanolic extract from the seed part of Trachyspermum ammi are summarized in **Table 2** and the data represented that the seeds of Trachyspermum ammi showed remarkable in vitro anticancer potential in the range of 70-100% against four human cancer cell lines.

Figure 1: *Nigella sativa* (Kalonji)

Figure 2: *Trachyspermum ammi* (Carom seeds)

The extract showed 100% growth inhibition on prostate cancer cells (PC-3), 90% growth inhibition on lung cancer cells (A-549), 80% growth inhibition on breast cancer cells (MCF-7) and 70% growth inhibition against colon cancer cells (HCT-116). However, the extract exhibited 31% growth inhibition (non-significant) against MIAPACA - a human cancer cell line from pancreatic origin, 21% growth inhibition (non-significant) against SHSY-5Y - a human cancer cell line from CNS origin and 09% growth inhibition (non-significant) against HEP-2 - a human cancer cell line from liver origin. Cancer is a disease that is uncontrollable and to a large extent, incurable. Cancer is a deadly disease facing the humanity today and this disease is responsible for 2-3% deaths worldwide annually. It has emerged as an important health problem in the developed / developing countries and recognized as the important cause of morbidity, mortality, disability in India also. According to World Health Organization's recent estimate, the cumulative death toll due to cancer will be 12 million by 2030. In India, around 5-8 lakhs of people are dying of cancer every year. Cancer cases related mortality on rise in J&K during past few years. The number of individuals living with cancer is continuing to expand, but most of the drugs used in cancer chemotherapy exhibit cell toxicity and can induce genotoxic, carcinogenic and teratogenic effects in non tumor cells. A number of side effects are related to currently available therapies for cancer treatment. Therefore, the research for alternative drugs of natural origin, which are less toxic, endowed with fewer side effects and more potent in their mechanism of action, is an important research line.

Keeping this in mind, the present investigation was carried out to evaluate the *in vitro* anticancer potential of kalonji and carom seeds 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 methanolic extracts from seed part of *Nigella sativa* (kalonji) and seed part of *Trachyspermum ammi* (carom seeds) showed significant *in vitro* cytotoxic activity against different human cancer cell lines .Kalonji displayed *in vitro* cytotoxic effect against two human cancer cell lines from two different tissues namely Breast (MCF-7), Liver (HEP-2) and the growth inhibition range was 80-97%.*Trachyspermum ammi* showed *in vitro* cytotoxic effect against four human cancer cell lines from four different tissues namely Breast (MCF-7), Colon (HCT-116), Lung (A-549), Prostate (PC-3) in the growth inhibition range of 90-100%. The active component possessing highest anticancer activity in *Nigella sativa* **(**black cumin)is thymoquinone and in general thymoquinone showed sustained inhibition of breast cancer cell proliferation with long-term treatment. Further, analysis using cell cycle assay showed the inhibition of the MCF-7 cell line proliferation at 25 µM concentration and led to S phase arrest significantly at 72 hrs treatment. G2 phase arrest was observed with increase in concentration of thymoquinone (50 µM)[8].The phytochemical analysis of extract (ethanolic) of *Trachyspermum ammi* (caromseeds) showed the presence of carbohydrates, tannins, alkaloids, flavonoids, phenols, glycosides, terpenes and steroids. The extract showed strong cytotoxic effect on MCF-7 breast cancer cell lines with an IC_{50} value of 25µg/ml. Further, the MCF-7 cells showed cell shrinkage, membrane blebbing and disorganized cell structures when treated with carom seed extract.[9]

5. Conclusion

Herbs and spices have long been used in culinary and medicinal preparations since time immemorial. Spices' delightful flavour and health benefits have made them essential part of human diet. Spices have enormous anticancer chemo preventive potential. Due to the presence of certain bioactive compounds in spices, they have potent antioxidant, anti-inflammatory and anticancer properties. Spices contain bioactive components such as alkaloids, terpenes, flavonoids, phenylpropanoids and anthocyanins which contribute to their therapeutic potential. Many of these spice-derived secondary metabolites have the ability to activate the cellular free radical scavenging system thus protecting against many metabolic syndromes. To conclude, isolation and characterization of active ingredients is required from these two spices that will surely serve as lead molecules for the development of anticancer drugs to provide a great promise and service to cancer patients.

6. Source of Funding

None.

7. Conflict of Interest

None.

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