

## **Chemopreventive activity of some spices against selected cell line**

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### **ABSTRACT**

*Herbs and spices have been traditionally used for prevention and cure of many diseases. Hence, the present study was aimed to investigate the effects of aqueous and alcoholic extract of four different spices (asafoetida, ginger, cinnamon and cardamom) extracts on human breast adenocarcinoma cell line (MCF) and HEP-G2 cancer cell lines through in-vitro growth inhibitory assay. Both aqueous and alcoholic extracts of spices (asafoetida, ginger, cinnamon and cardamom) showed significant activity as cytotoxicity agents for tumor cells. A significant decrease in MCF and HEP-G2 cell population by crude extract was observed. Among the spices assayed for their chemopreventive potential, asafoetida extract has shown the maximum inhibitory effect while cinnamon extracts showed minimum yet significant inhibitory action. Therefore, these spices might be used for natural healing of the tumor.*

**Keywords,** Chemopreventive activity, human breast adenocarcinoma cell line (MCF), HEP-G2, Spices.

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### **INTRODUCTION**

Since ancient times, plants have been utilized as an important source of medicines as they are a reservoir of chemical agents with antimicrobial properties. Herbal medicines are increasingly used as dietary supplements for treatment against different human disorders. Spice plants are renewable raw materials. Spices can be defined as "any dried, fragrant, aromatic or pungent vegetables or plant substances in whole, broken or ground forms, that contribute flavour, whose primary function in food is seasoning rather than nutrition and that may contribute relish or piquancy of foods and beverages" [1].

Herbs and spices have been used for thousands of years to enhance the flavour, colour and aroma of food. In addition to boosting flavour, herbs and spices are also known for their preservative [2] and medicinal value, which forms one of the oldest sciences [3] A large number of plants are used to combat different diseases [4,5] and possess antimicrobial activity [6,7,8,9,10,11,12,13,14]. Several spices particularly garlic, ajowain, black pepper, clove, ginger, cumin, cardamom, cinnamon, elaichi and caraway are used extensively in the Indian diet and in Indian medicine. Use of spices for medical benefits can be justified as these are easily absorbed

by our body and generally does not have any adverse effects.

The future of the natural habitat of medicinal plants is being threatened by ever increasing anthropogenic activities. Increased commercialization has resulted in over-harvesting of medicinally useful plants, which has diminished their number so they are now in danger of extinction. To overcome this alarming problem, the discovery of novel active compounds is the need of the day. Previously, all drugs and medicinal agents were derived from natural substances, especially from higher plants.

Considering the above discussion, chemopreventive activity of spice extracts i.e. asafoetida, ginger, cinnamon and cardamom were evaluated against MCF and HEP-G2 cancer cell lines.

## MATERIALS AND METHODS

### Spice samples and Extract Preparation

Asafoetida, ginger, cinnamon and cardamom were bought from the local market in Kanpur. Extracts were prepared by the method of Clarkson and Bibby, 1969[15]. Both water and alcohol extracts of spices were used. Water extracts were made by extracting 5 gm of ground spice in 100 ml distilled water in a Soxhlet extraction apparatus for four hours at 100° C. To prepare alcohol extracts 5 gm of ground spice was added to 100 ml of absolute alcohol and agitated at room temperature for eight hours in a wrist-action shaker. Thereafter, the mixture was allowed to stand for 12 hours, the alcohol evaporated without heat, and the residue was mixed with 100 ml of distilled water at 80° C.

### Cell Lines used

Human breast adenocarcinoma cell line (MCF) and HEP-G2 cancer cell lines were used for determination of anti-cancer effects of selected spices.

### Maintenance of Cell Culture

The cells at a density of  $1 \times 10^5$  from each cell line were transferred to MEM media and media was replaced after every 2 days until the outgrowth had spread to cover at least 50% of the growth surface. Further, the cells were sub cultured by enzymatic method using trypsin and maintained at MEM medium.

### In Vitro Growth Inhibition Assay

The sulforhodamine B assay was used according to the method of Skehan et al[16]. Cells were plated in a 96 well format ( $3 \times 10^4$  cells/well) tissue culture grade microtiter plate for twenty four hours. Spice samples were added to it and further incubated for 24 hours. DMSO was used as control. At the end of drug exposure, cells were fixed with 50% trichloroacetic acid and stained with 0.4% sulforhodamine B (Sigma-Aldrich), dissolved in 1% acetic acid (100µl/well) for 30 minutes, and subsequently washed with 1% acetic acid. Protein bound stain was solubilized with 150 µl of 10mM unbuffered Tris base, and cell density was determined using a colorimetric plate reader (wavelength 570nm). All samples were run in triplicate.

## RESULTS AND DISCUSSION

Chemopreventive activities of aqueous and alcoholic extracts of four commonly used spices in India were studied on human breast adenocarcinoma cell line (MCF) and HEP-G2 cancer cell lines. In vitro growth inhibition assay shows significant loss in cell viability of cancerous cell lines under study (Figure 1 & 2). All the extracts have showed maximum inhibition of cell

viability at a concentration of 20mg/ml. Asafoetida extract has shown the maximum inhibitory effect. The results for ginger and cinnamon have also shown promising results along with cinnamon extracts to be used for naturally healing cancer growth.

Alcoholic extracts have been shown to yield greater inhibitory action than their aqueous extracts. This could be due to the polyphenolic and flavonoid content of these extracts which have shown to have proven antioxidant and chemopreventive action.

The results could be validated with the report of Mallikarjuna *et al.* 2003 [17], they have thoroughly investigated the modulatory influences of asafoetida (*Ferula asafoetida L.*) on the mammary epithelial tissue differentiation, hepatic drug metabolizing enzymes, antioxidant profiles and N-Methyl-N-nitrosourea (MNU)-induced mammary carcinogenesis in Sprague-Dawley rats. It has also been reported that [6]-gingerol, an active ingredient of ginger (*Zingiber officinale*) has potent anti-angiogenic activity *in vitro* and *in vivo* [18]. These results point towards a possible role of [6]-gingerol in preventing cancers from becoming malignant, presumably by selective inhibition of angiogenesis formation at the tumor site. Ginger supplementation at the initiation stage and also at the post-initiation stages of 1,2-mimethylhydrazine (DMH)-induced colon carcinogenesis in male Wister rats has shown significant reduction in circulating lipid peroxidation and significant enhancement in the enzymic and non-enzymic antioxidants as compared to unsupplemented DHM-treated rats, thus suppressing the colon carcinogenesis in the presence of the procarcinogen DMH [19]. Masuda *et al.*[20] have determined the structures of more than 50 antioxidants isolated from the rhizomes of ginger and have reported [6]-Gingerol to possess substantial antioxidant and anti-inflammatory activity. Gingerol has also been reported to inhibit the growth of human colorectal cancer cells [21] and the development of mammary tumors [22] as well. Some compounds present in ginger may also exert cancer preventive effects by inducing apoptosis in cancerous or transformed cells. Studies suggest that some compounds present in ginger *e.g.* [6]-paradol *etc.* suppress proliferation of human cancer cells through the induction of apoptosis [23,24] and have been found to exert inhibitory effects on the viability of human HL-60 (promyelocytic leukemia) cells [24].

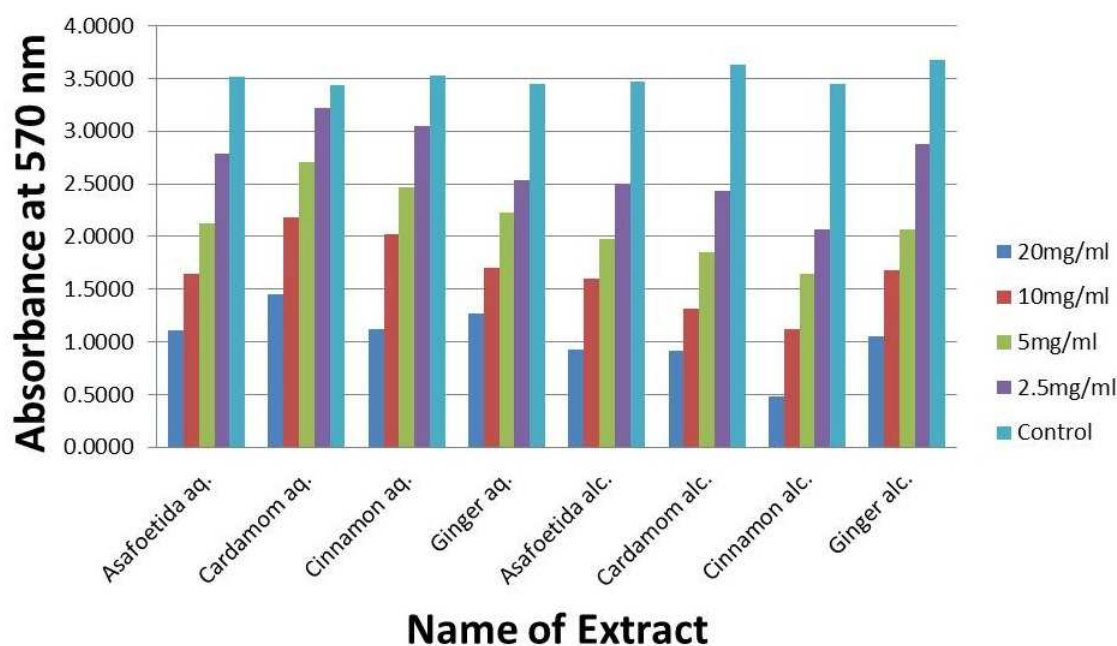
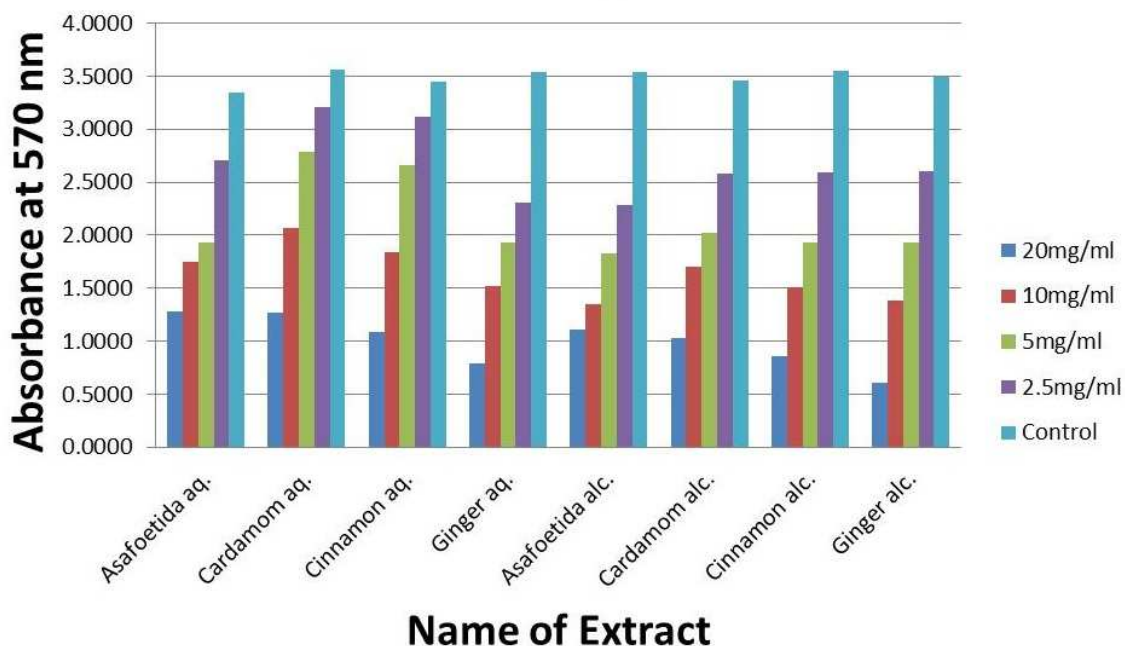


Fig 1. Results of in-vitro growth inhibition assay of spice extracts on MCF Cell Line



**Fig 2. Results of in-vitro growth inhibition assay of spice extracts on HEP-G2 Cell Line**

Some of the constituents of Cinnamon have proven value against bacteria and fungi, including the molds that produce the carcinogenic aflatoxins [25,26,27,28].

Cardamom has been reported to modulate the status of proliferation and apoptosis, and its role in altering cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expression has been confirmed [29]. These results suggest that aqueous suspensions of cardamom have protective effects on experimentally induced colon carcinogenesis [29].

## CONCLUSION

Herbs and spices are well known for their preservative and medicinal value, which can be investigated in discovering novel chemopreventive compounds. The present study was aimed to investigate the effects of aqueous and alcoholic extract of four different spice (asafoetida, ginger, cinnamon and cardamom) extracts on MCF and HEP-G2 cancer cell lines. Spice extracts were used for In-vitro growth inhibition assay on various selected cancerous cell lines that exhibited significant inhibitory activity of these spice extracts. A significant decrease in MCF and HEP-G2 cell population by crude extract was observed indicating the fact that asafoetida, ginger, cinnamon and cardamom can be used as chemopreventive agents against tumor cells.

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