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IN VITRO CYTOTOXICITY AND CELL VIABILITY ASSAY OF *CORIANDRUM SATIVUM* L. SEED POWDER EXTRACTS

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ABSTRACT

The anticancer activities of aqueous and ethanol seed powder extracts of *Coriandrum sativum* L. was investigated *in vitro* by MTT assay on human breast adenocarcinoma cell lines (MCF-7). Cells were exposed to various concentrations of both extracts for 72 hours. The percent cell viability increased with increase in concentration of the plant seed extracts after addition. The MTT assay evinced that both aqueous and ethanol seed powder extracts of *C. sativum* L. exhibited the *in vitro* cytotoxicity on MCF-7 human cell lines. The inhibition of cancer cell growth was highest in the 500 µg/mL concentration. The IC 50 values were better in the ethanol extract than in aqueous. It also induced cell death in MCF-7 cells. These results indicate that *Coriandrum sativum* L. seed powder extract could be used as potent anticancer natural medicine and could lead to drug formulation.

KEYWORDS: Anticancer, *Coriandrum*, Cytotoxicity, MCF-7, MTT.

INTRODUCTION

Traditional herbal medicine is gaining more popularity and is still widely practised. This has attracted the attention of many researchers and encouraged them to screen plants of medicinal interest in order to study the biological activities of their bioactive compounds (Bakkali *et al*., 2008). Breast cancer is second most common in women and accounts for 23% of all occurring cancers in women. Patients with breast cancer have increasingly shown resistance

and high toxicity to chemotherapeutic drugs. Plant-derived products have proved to be an important source of anti-cancer drugs.

The genus *Coriandrum sativum* L. is an annual herb of the family Apiaceae. It originates from the Mediterranean countries and now-a-days widely grown in India, Italy, America, Africa, Morocco. Coriander is also known as cilantro, Arab parsley, Mexican parsley, Dhanya (Mohamed Ramadan *et al.*, 2002). The use of coriander dates back to around 1550 BC, and it was one the oldest spice crops in the world. Medicinally, it was used as stimulant, aromatic and carminative (Coskuner and Karabala, 2007). It is widely used in folk medicine as spasmolytic, digestive, galactagogue and anti-microbial (Kasra Maroufi *et al*., 2010).

MATERIALS AND METHODS

Collection and Preparation of plant material

Seeds of *Coriandrum sativum* were procured from local market in Thiruvallur district. The collected seeds were dried under shade for 2-3days. The dried seeds were crushed gently to make a powder. 20g of seed powder was extracted with 100 mL of ethanol and distilled water respectively, for 24 hours in rotary shaker (Prashant *et al*., 2011).

Preliminary phytochemical screening

The aqueous and ethanol solvents extracts were subjected to the phytochemical screening using standard procedure for the detection of various phytochemicals (Harborne, 1998).

In vitro anti cancer activity by MTT assay

The aqueous and ethanol extracts of *Coriandrum sativum* was studied for *in-vitro* cytotoxicity using the MCF-7 cells (human breast cancer cell). Cell viability and cytotoxicity was tested by MTT assay in the presence of different concentration of both aqueous and ethanol extract. The cells were seeded in 96-well plates. Four well of each concentration were seeded and triplicate plates were used. Then, the cells were incubated at 37° C. After 36 hours of incubation various concentration of both aqueous and ethanol were added to the wells to obtain the final concentration of 18.75, 50, 100, 150, 300 and 500 µg/ml.

Controls were mixed with DMSO to obtain a final concentration of 1%. The cells were incubated for an additional 48 hours. 50 microliter of MTT (3-(4, 5 dimethylthiazol-2yl)-2, 5-diphenyl tertrazolium bromide)1mg/ml in phosphate buffer saline (PBS) was added to each well, and incubated at 37° C for 4 hours. The medium was removed and formazon was

dissolved in DMSO and the optical density was measured at 590 nm in a micro plate reader. (Rubinstein *et al*., 1990). The concentration for 50% inhibition of cell viability (IC50) was determined graphically. Percentage of residual cell viability was determined as:

Cell viability % = OD of treated cells / OD of control cells X 100

RESULTS AND DISCUSSION

The phytochemical screening indicated the presence of major phytochemicals such as Phenols, saponins and terpenoids with little presence of alakloids and tannins (Table 1).

Table. 1. Phytochemical screening of *C. sativum* seed powder extracts.

Phytochemicals	Name of the tests	Ethanol	Aqueous (Distilled water)
Alkaloids	Mayer's test	+	+
Phenol	Ferric chloride test	+++	++
Terpenoid	Salkowski test	++	+
Saponin	Foam test	+++	+++
Tannin	Braemer's test	+++	+

The various concentration of both aqueous and ethanol seed extracts used are given in table 2. The decrease in cell count was observed with increase in concentration of extracts. *In vitro* exposure of MCF-7 cells with various concentration of aqueous and ethanol extracts of coriander seeds suppressed the MCF-7 cancer cell growth. The inhibition of cancer cell growth was highest in the 500 µg/ml concentration with no viable cells present. The IC50 value was notable in the ethanol extract than in aqueous. It is also increased the death cells in the MCF-7 cells. This is contributed by active phytochemicals such as alkaloids, phenols and flavonoids found in the extracts (Table 1).

Table. 2: Percent cell viability of aqueous and ethanol seed extract of *C. sativum* on MCF-7 Cell line.

S. No	Concentration (µg/mL)	CELL VIABILITY (%)	
		Aqueous seed extract of *C. sativum*	Ethanol seed extract of *C. sativum*
1	Control	100	100
2	18.75	82.22 ± 0.15	78.40±0.41
3	50	65.02±0.18	50.14±0.21
4	100	32.13±0.28	25.38±0.16
5	150	14.41±0.11	7.32±0.11
6	300	2.02±0.21	1.03±0.05
7	500	0.00	0.00
IC 50 value ± SD (µg/mL)		**78**	**50**

MTT assay is a universally accepted *in vitro* method for screening the drugs having cytotoxic activity. Morphological changes were observed after treatment with *C. sativum* seed extracts. The probable reason might be attributed to the inhibition of cell proliferation and finally killing the cells which was well implicated by the absence of Formozan crystals in the dead cells. The cell death suggests that both the seed extracts could be a good cytotoxic agent. Most anticancer therapeutics relies on induction of apoptosis for inducing cell death in cancer cells and eradication of tumors (Wong, 2011).

Homeostasis of the organs is maintained in normal physiological conditions by a dynamic balance between the rate of cell proliferation and the rate of programmed cell death (apoptosis). Failure to undergo apoptosis has been implicated in tumour development and resistance to cancer therapy. Hence, agents that can induce apoptosis in tumour cells have the potential to become suitable anticancer drugs. MCF-7, the breast cancer-derived cell line, is deficient of caspase-3 and is relatively insensitive to many chemotherapeutic agents. A significant number of MCF-7 cells with morphological alterations were observed following *C. sativum* seed extract treatment. Mitochondria have been reported to play a critical role in the regulation of apoptosis. The opening of mitochondrial membrane pores is followed by an increase in different apoptosis mediators and activation of downstream caspases. Our results could have been due to dose-dependent upregulation of caspase in MCF-7 cells after seed extract treatment confirming the possibility that apoptosis could have been probably mediated by the intrinsic pathway (Karim *et al*., 2014).

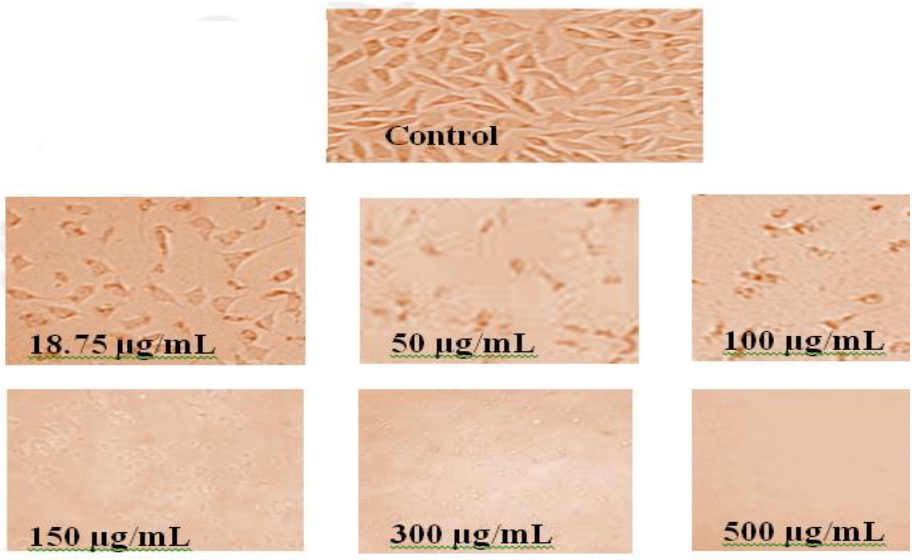

Fig. 1. Effect of *Coriandrum sativum* ethanol seed extracts on MCF-7 cells (morphological alterations and dose dependent decrease in cell counts).

Cytotoxic effect against the MCF-7 cell line is considered as a prognostic anticancer activity. Polyphenolic compounds might inhibit cancer cells by xenobiotic metabolizing enzymes that alter metabolic activation of potential carcinogens, while some flavonoids also alter the hormone production and inhibit aromatase to prevent the development of cancer cells. The mechanism of action of anti-cancer activity of phenols occurs by the disturbance of cellular division during mitosis (telophase stage). It was also reported that phenols reduce the amount of cellular protein and mitotic index and colony formation during cell proliferation of cancer cells. So they are regarded as promising anticancer agents against most human cancers (Ramya *et al*., 2017).

CONCLUSION

The results from the present study suggest that ethanol and aqueous seed extracts of *Coriandrum sativum* were able to induce cell growth inhibition in MCF-7 cell line. From this it is concluded that coriander seed powder extract can be used as potent anticancer natural medicine for the development of novel chemopreventive or chemotherapeutic formulations with reduced side effects.

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