

EVALUATION OF *INVITRO* ANTI-OXIDANT AND ANTICANCER ACTIVITY OF *CORIANDRUM SATIVUM* AGAINST HUMAN COLON CANCER HT- 29 CELL LINES

T. G. NITHYA^{1*}, D. SUMALATHA²

¹Department of Biotechnology, Faculty of Science and Humanities, SRM University Kattankulathur, India, ²Department of Biotechnology, Valliammal College for Women, Chennai, India. Email: nithyacan24@gmail.com

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ABSTRACT

Objective: The main aim of the study was to screen the ethanolic extract of *Coriandrum Sativum* leaves for its *invitro* anti-oxidant and anticancer activity and find its efficacy against H-29 Cell lines.

Methods: Ethanolic extract of *Coriandrum Sativum* were prepared and assayed for the presence of phytochemicals. *In vitro* antioxidant assay were performed by DPPH radical assay and FRAP assay. The effect of ethanolic extracts on HT-29 cancer cell lines were evaluated by MTT colorimetric assay.

Results: The preliminary phytochemical screening of ethanolic extract showed the presence of significant secondary metabolites. The efficacy of *Coriandrum Sativum* against HT-29 cell lines showed that the incubation of cancer cells reduced the viability of HT-29 cell lines and the dead cells were significantly increased with high extract concentration. Hence Ethanolic extract of *Coriandrum Sativum* exhibited high cytotoxicity.

Conclusion: Even at very low concentration *Coriandrum Sativum* showed high efficacy. In conclusion *Coriandrum Sativum* possess significant antioxidant activity and anticancer activity.

Keywords: *Coriandrum Sativum*, Anti oxidant, Anticancer, Colon cancer, HT 29 cell lines.

INTRODUCTION

Currently, one in four deaths in the United States are due to cancer [1]. When ranked within age groups, cancer is one of the five leading causes of death amongst both male and female and the single largest cause of death worldwide [1]. Cancer is a group of diseases initiated by loss of cell cycle control. Cancer is associated with abnormal uncontrolled cell growth [2]. Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). Indeed, the struggle to combat cancer is one of the greatest challenges of mankind [3]. This growing trend indicates efficiency in the present cancer therapies which include surgical operation, radiotherapy and chemotherapy. Since the average survival rates have remained essentially unchanged despite of such aggressive treatments, there is a critical need for anticancer agents with higher efficacy and less side effects that can be acquired at an affordable cost [4]. Chemoprevention is recognized as an important approach to control malignancy and recent studies have focused on the search for desirable chemopreventive agents. Natural products, particularly dietary substances, have played an important role in creating new chemopreventive agents [5]. According to Cragg and Newman over 50 % of the drugs in clinical trials for anticancer properties were isolated from natural sources [6]. Several natural products of plant origin have potential value as chemotherapeutic agents. Some of the currently used anticancer agents derived from plants are podophyllotoxin, taxol, vincristine and camptothecin [7]. The areas of cancer and infectious diseases have a leading position in utilization of medicinal plants as a source of drug discovery. Among FDA approved anticancer and anti-infectious drugs, drugs from natural origin have a share of 60 % and 75 % respectively [8].

Anticancer drugs discovered from herbal medicines have a long history and some of them have been used in clinical setting as a conventional anticancer drug [9]. Colorectal cancer, also known as colon cancer or bowel cancer is a cancer caused by uncontrolled cell growth in the colon or rectum, or in the appendix. Symptoms of colorectal cancer typically include rectal bleeding and anemia which are sometimes associated with weight loss and changes in bowel habits. Colorectal cancer is the third most commonly diagnosed cancer in the world, but it is more common in developed countries. Around 60% of cases were

diagnosed in the developed countries. It is estimated that worldwide, in 2008, 1.23 million new cases of colorectal cancer were clinically diagnosed, and 608,000 people died of the disease [10]. *Coriandrum sativum* is a medicinally important herb mainly from Asian origin possessing vast number of therapeutic applications [Table 1]. Coriander is used in the preparation of many household medicines to cure bed cold, seasonal fever, nausea, vomiting, stomach disorders and also used as a drug for indigestion, against worms, rheumatism and pain in the joints. Many of healing properties of coriander can be attributed to its exceptional phytonutrients and hence, it is often referred to as store house for bioactive compounds [11]. HT-29 is a human colorectal adenocarcinoma cell line with epithelial morphology. These cells are sensitive to the chemotherapeutic drugs 5-fluorouracil and oxaliplatin, which are standard treatment options for colorectal cancer. In addition to being a xenograft tumor model for colorectal cancer, the HT-29 cell line is also used as an *in-vitro* model to study absorption, transport, and secretion by intestinal cells [12]. Therefore, in the present study we analyzed *Coriandrum sativum* leaves for the presence of bioactive components and also evaluated *invitro* anti cancer activity against HT-29 cell lines.

MATERIALS AND METHODS

Collection of Sample

Coriandrum Sativum leaves were collected from Chennai, Tamilnadu (India) and authenticated. The sample was air dried.

Preparation of Extract

The leaves were air dried and powered with a mechanical grinder, passing through sieve and stored in a tight container. Then 25gms of air dried powder was continuously refluxed with ethanol at 45°C for 3hrs using soxhlet apparatus [13]. The mixtures were filtered. The filtrates were evaporated using vacuum rotary evaporator & air dried at 40°C.

The stock solution of crude ethanolic extract were prepared by diluting the dried extracts with 0.25% dimethyl sulphoxide (DMSO) solution to obtain a final concentration of 100mg/ml.

Phytochemical screening : The qualitative tests were carried out in the ethanolic extract of *Coriandrum Sativum* using standard procedures [14-16]. The extract were analysed for the presence of significant secondary metabolites viz Flavonoids, Alkaloids, Saponins, Cardiac glycosides, Tannins, Anthraquinone and Steroids [Table 2]

Table 1: Taxonomy of Plant

Kingdom	Plantae
Sub-kingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Apiales
Family	Apiaceae
Genus	Coriandrum L.
Species	Coriandrum sativum L.

Table 2: Phytochemical Analysis of *Coriandrum Sativum*

Compounds	Analysis
Tannins	+
Saponins	--
Flavonoids	--
Alkaloids	+
Steroid	+
Anthraquinone	--

+ Present -- Absent

In vitro Antioxidant Assay

DPPH Radical Assay

DPPH test is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517nm and also for a visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance.

The ability of the extracts to scavenge DPPH free radicals were determined by standard method [17]. Different concentration of the test sample ranging 25 -125 µg/ml were placed in a cuvette and 0.5ml of 100mM methanolic solution of DPPH was added. Mixtures were vigorously shaken and allowed for 30 min incubation in dark. Ascorbic acid was used as control. The absorbance was then measured at 517nm. Inhibition percentage of DPPH radical was calculated using the following formula

$$\text{DPPH radical \%} = \frac{(1 - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

FRAP Assay

(a) 3.1g of sodium acetate trihydrate was weighed and to that 16 ml of glacial acetic acid were added and made up to 1 liter using distilled water. (b) 10 mM of TPTZ (2, 4, 6-tripyridyl-*s*- triazine) dissolved in 40mM HCl. (c) 20 mM of FeCl₃.6H₂O. were prepared

The working FRAP reagent was prepared by mixing a b & c in the ratio of 10:1:1 at the time of use. Ascorbic acid was used as control [18]. The ethanolic extract of *Coriandrum Sativum* was dissolved in methanol in 0.5mg/ml concentration. 4.5ml of freshly prepared FRAP reagent was added to the prepared extract and stirred well. After 5 mins, absorbance was measured at 593 nm using FRAP working solution as blank [19]. The relative activity of the sample was compared with ascorbic acid. FRAP value was calculated using the formula

$$\text{FRAP value} = \frac{\text{Change in absorbance of sample from 0 - 4 mins}}{\text{Change in absorbance of standard from 0 - 4 min}} \times \text{FRAP value of Std}$$

In vitro Cytotoxicity Assay

Cell culture and experiment design

HT-29 cell lines were obtained from King's Institute, Chennai. The cells were grown and maintained in a humidified incubator at

37°C under 5% CO₂ atmosphere in MEM medium (Minimal Essential Media) supplemented with TPVG & 10% Fetal calf serum and (100 units/ml penicillin). For experimental purpose cells were plated in 48 well plates (at a density of 1x10⁴ cells/ml). After 25hrs incubation, to allow cell attachment, the cells were treated with fresh medium containing different concentration of ethanolic extract of *Coriandrum Sativum* ranging from 10-100 µg/ml, dissolved in DMSO and incubated for 48hrs.

MTT Assay

It is a sensitive, quantitative and reliable colorimetric assay that measure viability, proliferation and activation of cells. The assay is based on the capacity of the cellular mitochondrial dehydrogenase enzyme in living cells to reduce the yellow water-soluble substrate 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into a dark blue/purple formazan product which is insoluble in water. The amount of formazan produced is directly proportional to the cell number in a range of cells lines [20-21]. At the end of 48hrs incubation, the medium in each plate containing the ethanolic extract of *Coriandrum Sativum* was added with 200µl of MTT solution and incubated for another 4hrs. The supernatant was then removed & replaced with 500µl of DMSO to dissolve the resulting MTT formazan crystals followed by mixing & measuring the absorbance at 590nm.

$$\text{Cell viability \%} = \frac{\text{OD of sample}}{\text{OD of control}} \times 100$$

RESULTS

Ethanolic extract of *Coriandrum Sativum* was screened for the presence of Phytochemicals and showed the presence of bioactive compounds like Alkaloids, Flavonoids, Tannins, Saponins, and Cardiac glycosides. DPPH assay showed that *Coriandrum Sativum* showed high scavenging activity with 60.14% inhibition [Fig. 1]. FRAP values of the extract indicates that reducing power of the extract increased with concentration [Fig., 2]. The efficacy of ethanolic extracts on HT-29 cancer cell lines were evaluated by MTT assay. Values of percentage viability of treated cells were plotted against extracts concentration. The present study demonstrated that the antioxidant and anticancer profile of *Coriandrum Sativum* was effective against HT-29 cells. MTT assay showed that the incubation of cancer cells lines with *Coriandrum Sativum*, reduced the viability of cancer cells and the dead cells were significantly increased with extract concentration. Thus the ethanolic extract of *Coriandrum Sativum* exhibited high cytotoxicity of 93.8% [Fig. 3].

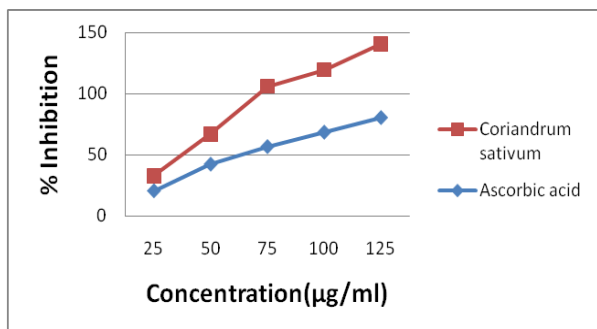


Fig. 1: Free radical scavenging activity of Coriandrum Sativum

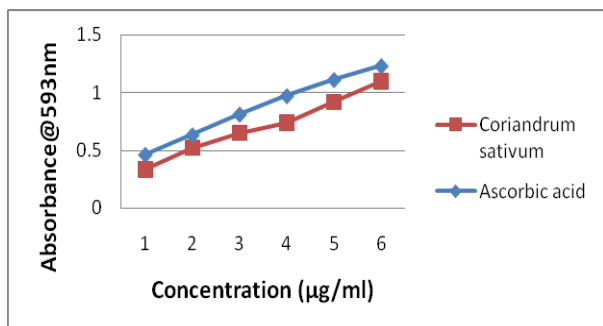


Fig. 2: Antioxidant activity measured by FRAP assay

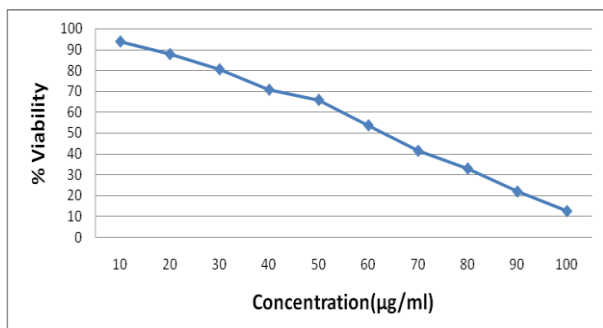


Fig. 3: Anticancer activity of Coriandrum Sativum on HT-29 Colon cancer cell lines

DISCUSSIONS

Medicinal plants constitute a common alternative for cancer prevention and treatment in many countries around the world [22-23]. Extracts of certain medicinal plants are believed to contain a wide array of polyphenolic compounds which might possess cancer preventive and therapeutic properties [24]. Although numerous drugs are currently in use for cancer chemotherapy, they exhibit cell toxicity, induces genotoxic, carcinogenic, and teratogenic effects in non-tumor cells[25]. These side effects limits the use of chemotherapeutic agents despite of their high efficacy in treating target malignant cells. Therefore, the search for novel drugs that are both effective and non-toxic bioactive plant products has been increased[26]. Our goal was to determine whether the extracts of *Coriandrum Sativum* exerted an inhibitory effect on cancer cell proliferation and caused cell death. The present study observed that the ethanolic extract of *Coriandrum Sativum* inhibits the proliferation of HT 29 Human Colon cancer cell lines. The cytotoxicity effect was highest with increase in concentration. The cytotoxicity was concentration-dependent and cell line specific. This clearly indicates the presence of potent bioactive principles in the crude extract that might be useful as antiproliferative and antitumor agents.[27] Although the mechanism of the action have not been elucidated, it was understood that the extract contains flavonoids and antioxidant polyphenolic compounds.[28] These compounds are known to scavenge the formation of free radicals, and have great potential in ameliorating cancer cells.[29].

CONCLUSION

The results of the present study reveals the potentiality of *Coriandrum Sativum* against colon cancer cell lines and supports the need of further studies to isolate it as an potential anticancer drug. In future study, the isolated principles from *Coriandrum Sativum* needs to be evaluated in scientific manner using scientific experimental animal models and clinical trials to understand exact molecular mechanism of action, in search of lead molecule from natural resources. Additionally, the study supports the anticancer property of medicinal plants used in the traditional Indian medicine system and further evaluation of the selected medicinal plants for an effective anticancer drug with minimal side effects. Therefore supplementing a balanced diet with *Coriandrum Sativum* leaves may have beneficial effect in treating colon cancer.

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