Evaluation of Biological Activities of Seeds of *Coriandrum Sativum*

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Abstract:*In vitro*, antioxidant, anticancer, cytotoxic and antiviral activities of *C. sativum* seeds was investigated. To detect the antioxidant activity of methanol and hexane extracts of *C. sativum* seeds, assays to demonstrate its reducing power and total phenolic content was performed by spectrophotometry. The cytotoxic and anticancer effect of methanol, hexane and aqueous extracts was assessed using MTT assay. Additionally, the antiviral activity of methanol, hexane and aqueous extracts against HSV-1 was investigated by plaque reduction assay.Based on absorbance readings, the reducing power of methanol and hexane extracts of *C. sativum* seeds were approximately equal, while the absorbance readings indicated that the total phenol content assay of methanol extract was higher than that of the hexane extract. Based on MTT assay, both hexane and aqueous extracts exhibited inhibition of vero cells with CC_{50} of 600 µg/ml and 700 µg/ml, respectively, while the minimum inhibition of HepG2 cells was observed at a concentration of 350 µg/ml for the three extracts. The aqueous and hexane extracts exhibited reductions in the formation of HSV-1 plaques with an observed IC_{50} of 350 µg/ml and 250 µg/ml, respectively.Based on the results from this study, the hexane, methanol and aqueous extracts of *C. sativum* seeds demonstrated anti-cancer and antiviral effects while the hexane and methanol extracts demonstrated antioxidant potency.

Key Words: Anticancer, Antiviral, MTT assay, Reducing power, Total phenolic content

1. Introduction

Apiaceae (Umbelliferae) is a large family of flowering plants [1]. It comprises of 455 genera distributed primarily in temperate regions. *Coriandrum sativum L.* (Apiaceae) also referred to ''cilantro" is an annual plant native to the Mediterranean and Middle Eastern regions [2]. This plant is of economic importance since it is used as a flavoring agent in food products, perfumes, cosmetics and drugs. In addition to its culinary value, coriander is known for its wide range of healing properties. It is generally used in gastrointestinal complaints such as anorexia, dyspepsia, flatulence, diarrhea, griping pain and vomiting [3]. The seeds are used in traditional medicine as nematicidal, antibacterial and larvicidal agents [4, 5]. The essential oil (1%) and various extracts obtained from Coriander have been shown to possess, antioxidant, anxiolytic, antidiabetic, anticancerous, antimutagenic and free radical scavenging activities [6]. Oxidative stress results from the imbalance between reactive oxygen species production and inactivation. Oxidative stress has been implicated in a variety of disorders such as cancer, Parkinson's disease and AIDS. Furthermore, increased levels of products of lipid peroxidation such as malondialdehyde and of oxidative DNA damage such as 8-hydroxyguanine have been observed in HIV-positive indivuals [7].More recently, there have been advocacies for the use of the green parts of this plant as an antioxidant agent, to the best of our knowledge, there have been no published reports on biological properties (anticancer, antiherpetic and cytotoxic properties) of the plant seeds. Therefore, this study was conducted to investigate the biological properties of C. sativum. The aims of this study are as follows: (i) to determine the antioxidant properties of C. sativum seeds (ii) to evaluate the anticancer activity of the seeds (iii) to evaluate its antiherpetic activity as a selective agent.

2. Materials and Methods

Coriander seeds powder was purchased from the local market in Hentian Kajang, Bangi, Malaysia. Methanol and n- hexane of analytical grade were supplied by Chemo Lab, Malaysia, while ascorbic acid and gallic acid were purchased from High Valley Holistic Nutrition Sdn. Bhd. A 0.2 M sodium phosphate buffer (pH 6.6), 2.5 ml of 1% potassium ferricyanide (Summit Chemical Industrial Co. Limited), 2.5 ml of 10% trichloroacetic acid (Prospect Ace Sdn. Bhd., Malaysia), 0.1% ferric chloride solution, Folin–Ciocalteu reagent (Sigma – Aldrich), 20% Na_2CO_3 solution (Industries Sdn Bhd) were prepared and used. Vero, hepatocarcinoma (HepG2) cell lines (Shahrul lab, UKM), non-essential amino acids (Thermo Fisher Scientific Brand, Malaysia), (MTT (3-[4,5-dimethyl thiozole-2-yl] -2-5-diphenyl tetrazolium bromide) and methyl cellulose(MC) (Sigma – Aldrich) were used in the cell culture. Also, an incubator (Shel Lab, USA), centrifuge (VWR International, USA), a microplate ELISA reader (Bio-Rad, Singapore) and spectrophotometer (Max Lab Technology (M) Sdn Bhd) were used in this study

2.1 Preparation of Plant Extract:

The extraction of the *coriander seeds* was carried out using known standard procedures. The powder of the plant material was initially extracted with methanol, hexane and aqueous solvents.

2.2 Antioxidant Assays:

2.2.1Reducing Power:

In this assay, the yellow color of the test solution changes to green depending on the reducing power of the test

specimen. The presence of reductants in the solution causes the reduction of the Fe^{3+}/ferric cyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by the measurement of the absorbance at 700 nm [8, 9]. The method of Raja and Shaker [9] was used to assess the reducing power of coriander seeds extract. One milliliter of different concentrations of each seed extract and ascorbic acid (10, 25, 50, 75 and 100 1mg/mL) in distilled water was mixed with 2.5 ml of a 0.2 M sodium phosphate buffer (pH 6.6), and 2.5 ml of 1% potassium ferricyanide and incubated in an incubator at 50 ^{O}C for 20 min. After cooling, 2.5 ml of 10% trichloroacetic acid was added to the mixture and was centrifuged at 3000 rpm for 10 min. The supernatant (1.25 ml) was then mixed with 1.25 ml distilled water and 0.25 ml of 0.1% ferric chloride solution. The intensity of the blue–green color was measured at 700 nm. Ascorbic acid was used as the positive control. Tests were carried out in duplicates.

2.2.2 Total Polyphenol Content:

The total polyphenol was assayed colorimetrically by using the Folin–Ciocalteu reagent, following Rajamanikandan et al [10] and Sidker et al [11]methods with slight modification. An aliquot (50 µl) of a suitable diluted methanolic seed extract was added to 2 ml of distilled water and 0.300 ml of the Folin–Ciocalteu reagent. The mixture was allowed to stand for 5 min before adding 0.8 ml of 20% Na_2CO_3 solution. Then, the solution was adjusted with distilled water to 5 ml and mixed thoroughly. After incubation for 30 min at 25 ^{O}C, the absorbance versus prepared blank was read at 765 nm. Gallic acid was used as the positive control and the calibration curve range was 25–250 mg/mL (R2 =0. 99). All analysis were performed in duplicates.

2.3 Cell Lines and Cultural Conditions**:**

Vero and hepatocarcinoma (HepG2) cell lines, were cultured in DMEM medium with 5% and 10% FBS, penicillin/streptomycin (100 µ/L) and non-essential amino acids under a fully humidified atmosphere, 5% CO_2 at 37° C

2.3.1 MTT Test:

The effect of seed extracts of *C. sativum*on the viability of Vero cells and the inhibition of HepG2 cells were determined by MTT (3-[4,5-dimethyl thiozole-2-yl]-2-5-diphenyl tetrazolium bromide) assay [12, 13]. Briefly, 100µL of each vero and HepG2 cell suspensions in growth medium were plated in 96-well microtitre plates at concentrations of $1x10^5$cells/well and incubated for 24h at 37° in a humidified incubator. The growth medium was exchanged with 100µL of the test sample. Test samples were prepared by dissolving extracts in DMSO (stock 1mg/mL) followed by diluting with distilled water to give a final concentration of 90, 100, 200, 300, 500, 600 and 700 µg/mL. Different concentrations of the extracts were added to the respective wells in such a way that final volume in each well is 100µL for vero and 200µL for HepG2 cells. Each concentration was placed in triplicate. The plates were incubated for 72h. Subsequently, 5mg/mL phosphate buffer solution), 10µl of MTT was added to each well of the plate. The plates were incubated for 3h at 37^{O}C, then 100µL of DMSO was added to dissolve formazone. The plates were shaken for 15 min. Optical density (OD) was measured on a microplate ELISA reader at 540 nm with cells and DMEM as a control. The cytotoxicity was obtained by comparing the absorbance between the samples and control. The percentage viability was calculated as follows:

% viability = OD of treated cells/OD of control cells x100

% inhibition = 100 - % viability

2.3.2 Antiviral Activity:

Antiviral assay was carried out according to Cheng, Lin [14] method with some modification. Vero cell line (2×10^5 cells/ mL was prepared in 24 wells (500 µL in each well). When the cells reached between 80-90% confluency, the medium was removed from the wells. HSV-1 at 50 PFU and DMEM were added subsequently to the cells and incubated for 1 h. The medium was removed and different concentrations of crude extract of methanol, hexane and aqueous of *C. sativum*(concentration is less than the CC_{50} values) were added to the methyl cellulose(MC) which was applied to the cells. This was then incubated for two days. After the plaques appeared, the plates were stained with crystal violet and shaken for about 30 min, followed by gentle washing. The plates were left to dry and the number of plaques was counted using an inverted microscope. The IC_{50} was defined as the concentration of the extracts that inhibit 50% of the plaques in virus-infected cells compared to control. The mean number of plaques was used to calculate the percentage of plaques inhibited. A curve relating percent plaque inhibition against extract concentrations was used to calculate the inhibitory concentration that elucidated a 50% reduction of cell viability (IC_{50}). The experiments were performed in triplicates with the mean values represents three experiments

2.4 Statistical Analysis:

Data were given as mean ± S.D of two independent experiments. All statistical analysis was conducted using Microsoft Excel.

3. Results & Discussion

Antioxidant Activity:

3.1 Reducing Power Assay:

Results for reducing power of the methanol, hexane extracts and standard are presented in Table 1. The reducing power increases with the increase in the amount of sample and standard concentrations and shows a good linear relation in the standard (R^2=0. 9938) and sample

extracts (R^2 = 0. 93). Reducing power is measured by the transformation of Fe^{3+} Fe^{2+} in the presence of extracts [15]

TABLE 1
ABSORBANCE OF STANDARD, METHANOL AND HEXANE EXTRACTS AT VARIOUS CONCENTRATIONS (MG/ML) IN FERRIC REDUCING POWER DETERMINATION

	Absorbance (µg/ml)		
Concentration	Ascorbic acid (standard)	Methanol	Hexane
10	0.086±	0.2±	0.217±
25	0.165±	0.21±	0.226±
50	0.367±	0.229±	0.232±
75	0.585±	0.237±	0.236±
100	0.828±	0.278±	0.254±

3.2 Phenolic Content

As shown in Table 2, the total phenol content shows good linear relation in both standard, as well as sample extracts Polyphenols activity is believed to be mainly due to their redox properties [18]. Phenolic compounds are also very important plant constituents because of their hydroxyl groups confer scavenging ability [19]

3.3 Cytotoxicity:

Both Table 3 and Fig.1 show the CC_{50} values of the hexane and aqueous extracts towards vero with the value of more than 20 µg/mL indicating that the samples were non cytotoxic[16, 17]. Thus, the antiviral activity is now worth to be demonstrated for the potential as antiviral agents

TABLE 3
CYTOTOXICITY (CC50), INHIBITION CONCENTRATION (IC50) AND SELECTIVE INDEX (SI) OF DIFFERENT EXTRACTS OF C. SATIVUM

Extract	CC_{50} (µg/ml)	IC_{50} (µg/ml)	SI
Aqueous Ex	700	350	2
Methanol Ex	nd	nd	nd
Hexane Ex	600	250	2.4

Ex = Extract, nd = not determined

100	0.132±	0.02±	0.011±
200	0.216+	0.029+	0.014+

3.4 HSV-1 Inhibition:

Table 3 and Fig. 2 summarize antiviral inhibition of the solvent extracts. Hexane and aqueous extracts displayed antiviral activity compared to methanol extract as 99 and 95% of inhibition were achieved at 500 µg/mL and associated IC*50* values of 250 µg/mL and 350 µg/mL respectively. On the other hand, the methanol extract produced no inhibition in the number of plaques at all tested concentrations. The selective index (SI) was used to associate the cytotoxicity and the antiviral potential of the tested extracts.

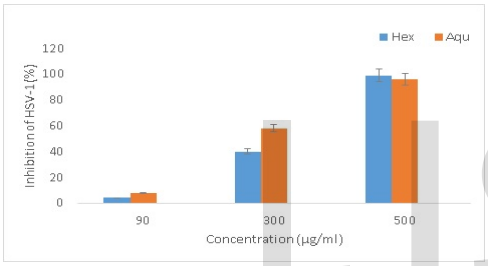

Fig. 2. The inhibition % of different extracts of *C. sativum* on HSV-1 , Hex = Hexane, Aqu = Aqueous

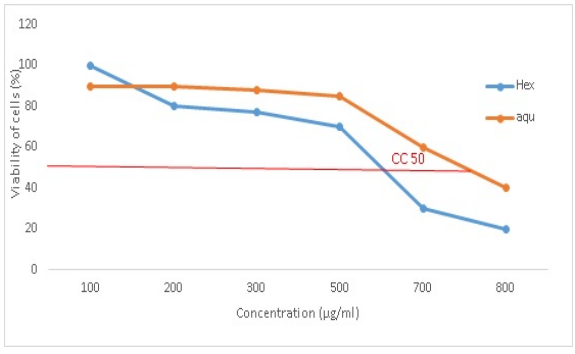

Fig. 1. Viability of cells of aqueous and hexane extracts of *C. sativum*, *Hex= Hexane, aqu = Aqueous*

Apiaceae species have been reported in previous studies. Essential oil of *H. anisactis*exhibited antibacterial activity against gram-positive bacteria and also *Escherichia coli* [20]. The methanol and dichloromethane extracts of *Bupleurum marginatum*demonstrated antitrypanosomal effect and moderate activity against *Streptococcus pyogenes* while the dichloromethane extract inhibited 73.6% of the plaque formation by hepatitis A virus. Also, CCRF-CEM cells were the most sensitive to both extracts and the cytotoxicity was mediated by induction of apoptosis [21]. A comparative evaluation of cytotoxicity and antiviral activity showed that kellerin that was isolated from the gum resin of Ferula assa-foetida had inhibited the viral titre of HSV-1 virus[22]. A study by Gomez-Flores, Hernández-Martínez [23] showed that *C. Sativum*seed aqueous and methanol extracts did not alter spleen lymphoproliferation, whereas *C. sativum*leaf aqueous extract stimulated significant ($p<0.01$) 14 to 45 percent spleen lymphocyte proliferation at concentrations ranging from 7.8 to 125 µg/mL, respectively. In addition, *C. sativum*(seed and leaf) aqueous extracts did not alter thymus lymphoproliferation. The methanol extract of *C. sativum*seed, did not alter thymus proliferation, whereas *C. Sativum* leaf extract caused significant ($p<0.01$) 44 to 59 percent proliferation at the concentrations tested whereas *C. sativum*seedmethanol extract showed high tumour cell toxicity.On the other hand, *C.sativum* was reported to protect against experimental colon cancer in rats [24], and its methanol extract was cytotoxic against the tumour cell lines MK-1, HeLa and B16F10 [25]. In addition, Chithra and Leelamma [24], study revealed that, the coriander crude extract was found to be efficiently demonstrate antitumor effects of colon cancer. Also, pthalide compounds found in coriander showed potential anticancer effects in another study [26].

On the other hand,Kumari and Ranjan [27],demonstrated that, the reducing power of plant extracts is highly

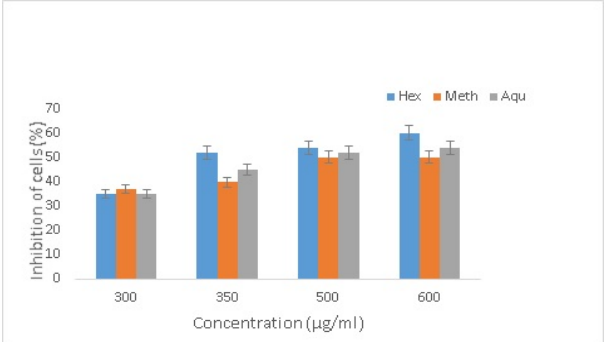

Fig.3. Graph showing differences in inhibition of Hep G2 cell line by seed extracts of *C. sativum*, , Hex = Hexane, Aqu = ,Aqueous, Meth= Methanol

phenolic contents. The antioxidant capacity observed could also have been due to the presence of some other phytochemicals such as ascorbic acid, tocopherol and pigments which interactsynergistically with phenolic contents to produce observed antioxidant activities. Again, phenolic compounds differ in their antioxidant activities depending on their structure. On the other hand, a study by Tang et al [29] revealed that the ethyl acetate extract of *C. sativum* roots had high anti-proliferative activity on MCF-7 cells and had high phenolic content, as well as FRAP and DPPH scavenging activities. Antioxidant and anticancer activities of *C. sativum* reported in literature mostly focus on the aerial parts of the herb. In this study, the antioxidant, anticancer, cytotoxic and antiviral activities of *C. sativum*seeds was investigated. The differences between our results and previously published literature on the subject could be attributed to variabilities in their growing geographic area, growth stage and collection season [20, 30]

4. Conclusion

The results from this study indicate that the seeds of *C. sativum*possess antioxidant properties and could serve as free radical inhibitors. The anticancer and antiviral properties of *C. sativum*are effective and can be used as easily accessible source in pharmaceutical industry

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Disclosure statement

The Authors declare that they have no conflict of interest to disclose

References

[1] Coskuner, Y. and E. Karababa, "*Physical properties of coriander seeds (Coriandrum sativum L.),*"Journal of Food Engineering. vol.2,no. 2, pp.408-416,2007

[2] Trifan, A., et al., "*Contributions to the chemical study of the essential oil isolated from coriander fruits (Sandra cultivar),*"Rev Med Chir Soc Med Nat Iasi. vol.4,no. 4, pp.1271-7,2011

[3] Jabeen, Q., et al., "*Coriander fruit exhibits gut modulatory, blood pressure lowering and diuretic activities,*"J Ethnopharmacol. vol.1,no. 1, pp.123-30,2009

[4] Sedaghat, M., et al., "*Larvicidal Activity of Essential Oils of Apiaceae Plants against Malaria Vector, Anopheles stephensi,*"Iran J Arthropod Borne Dis. vol.2,no. 2, pp.51-9,2011

[5] Moradian, H., et al., "*In vitro comparison of antimicrobial activity of aqueous decoction of Coriandrum sativum, and Dentol Drop with chlorhexidine on Streptococcus mutans,*"Iran J Microbiol. vol.3,no. 3, pp.239-43,2013

[6] K, M., et al., "*Changes on essential oil composition of coriander(Coriandrum sativum L.) fruits during three stages of maturity,*"Food Chemistry. vol.4,no. 4, pp.1131-1134,2007

[7] Singh, I.P., S.B. Bharate, and K. Bhutani, "*Anti-HIV natural products,*"CURRENT SCIENCE-BANGALORE-. vol.2,no. 2, pp.269,2005

[8] Pisoschi, A.M., "*Methods for total antioxidant activity determination: a review,*"Biochemistry & Analytical Biochemistry. 2011

[9] Raja, G. and I.A. Shaker, "*Phytochemical Screening and Reducing Power Assay of Nut Extracts of Juglans regia L,*"International Journal of Chemical and Life Sciences. vol.01,no. 01, pp.1026-1032,2012

[10] Rajamanikandan, S., et al., "*Radical Scavenging and Antioxidant Activity of Ethanolic Extract of Mollugo nudicaulis by Invitro Assays,*"Indian Journal of Pharmaceutical Education and Research. vol.4,no. 4, pp.310-316,2011

[11] Sikder, M., et al., "*In vitro antioxidant, reducing power, free radical scavenging and membrane stabilizing activities of Spilanthes calva,*"Bangladesh Pharm J. vol.1,no. 1, pp.63-67,2010

[12] Patharakorn, T., et al., "*Antibacterial activity and cytotoxicity of the leaf essential oil of Morus rotunbiloba Koidz,*"J. Med. Plants Res. vol.9,no. 9, pp.837-843,2010

[13] Zhang, L., et al., "*N-butanol fraction of Entada phaseoloides ethanol extract inhibits hepatocellular carcinoma HepG2 cell proliferation by inducing apoptosis,*"Journal Balkan Union of Oncology. vol.2,no. 2, pp.406-411, 2014

[14] Cheng, H.-Y., C.-C. Lin, and T.-C. Lin, "*Antiviral properties of prodelphinidin B-2 3'-O-gallate from green tea leaf,*"Antiviral chemistry & chemotherapy. vol.4,no. 4, pp.223-229,2002

[15] Kumar, H.K., et al., "*Studies on the free radical scavenging activity of Syagrus romanzoffiana,*"Int J Pharm. vol.2,no. 2, pp.81-84,2012

[16] Moo-Puc, R., D. Robledo, and Y. Freile-Pelegrín, "*In vitro cytotoxic and antiproliferative activities of marine macroalgae from Yucatán, Mexico Actividad citotóxica y antiproliferativa in vitro de macroalgas marinas de Yucatán, México,*"Ciencias Marinas. vol.4,no. 4, pp.345-358,2009

[17] Jain, R. and S.K. Jain, "*Screening of in vitro cytotoxic activity of some medicinal plants used traditionally to treat cancer in Chhattisgarh state, India,*"Asian Pacific Journal of Tropical Biomedicine. vol.2,no. 2, pp.S147-S150,2011

[18] Adedapo, A.A., et al., "*Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of Calpurnia aurea,*"BMC complementary and alternative medicine. vol.1,no. 1, pp.53,2008

[19] Bhalodia, N.R., et al., "*Evaluation of in vitro antioxidant activity of flowers of Cassia fistula Linn,*"International Journal of PharmTech Research. vol.1,no. 1, pp.589-599,2011

[20] Torbati, M., et al., "*Chemical composition and in vitro antioxidant and antibacterial activity of Heracleum transcaucasicum and Heracleum anisactis roots essential oil,*"Bioimpacts. vol.2,no. 2, pp.69-74,2014

[21] Ashour, M.L., et al., "*Anti-infective and cytotoxic properties of Bupleurum marginatum,*"Chin Med. vol.1,no. 1, pp.4,2014

[22] Ghannadi, A., et al., "*Anti-Viral Evaluation of Sesquiterpene Coumarins from Ferula assa-foetida against HSV-1,*"Iran J Pharm Res. vol.2,no. 2, pp.523-530,2014

[23] Gomez-Flores, R., et al., "*Antitumor and immunomodulating potential of Coriandrum sativum,*"Journal of Natural Products. 2010

[24] Chithra, V. and S. Leelamma, *"Coriandrum sativum--effect on lipid metabolism in 1,2-dimethyl hydrazine induced colon cancer,"*J Ethnopharmacol. vol.3,no. 3, pp.457-63,2000

[25] Nakano, Y., et al., *"Antiproliferative constituents in Umbelliferae plants II. Screening for polyacetylenes in some Umbelliferae plants, and isolation of panaxynol and falcarindiol from the root of Heracleum moellendorffii,"*Biol Pharm Bull. vol.3,no. 3, pp.257-61,1998

[26] Ganesan, P., et al., *"Comparative study of bioactive compounds in curry and coriander leaves: An update,"*Journal of Chemical & Pharmaceutical Research. vol.11,no. 11, 2013

[27] Kumari, M. and N. Ranjan, *"In vitro antioxidant activity of extract of bulb of Allium sativum Linn using DPPH and FRAP assays with evaluation of total phenolic content "*International Journal of Bioassays. vol.02,no. 02, pp.1752-1755,2014

[28] Vishwakarma, S., et al., *"Antioxidant Activities of Some Tuberous Plant Leaves,"*International Journal of Pharmaceutical Sciences Review & Research. vol.1,no. 1, pp.28-33,2013

[29] Rahiman, S., B.A. Tantry, and A. Kumar, *"Variation of antioxidant activity and phenolic content of some common home remedies with storage time,"*Afr J Tradit Complement Altern Med. vol.1,no. 1, pp.124-7,2012

[30] Tang, E.L., et al., *"Antioxidant activity of Coriandrum sativum and protection against DNA damage and cancer cell migration,"*BMC Complement Altern Med. pp.347,2013

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