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In Vitro Anticancer Activity of *Ocimum basilicum* Against Different Mammalian Cell Lines

K. Santhi[1], K. Durgadevi[1] and V. Ramamurthy*[3]

[1-3] P. G. & Research Department of Biochemistry, Maruthupandiyar College, Thanjavur - 613 403 (Affiliated to Bharathidasan University, Tiruchirappalli), Tamil Nadu, India

Abstract

Cancer is the second leading cause of death globally, and despite the advances in drug development, it is still necessary to develop new plant-derived medicines. Compared with using conventional chemical drugs to decrease the side effects induced by chemotherapy, natural herbal medicines have many advantages. In this study the anticancer potentials of plants were investigated against A549, HLC1 and PC14. Cytotoxicity of extracts was determined by MTT assay. The results showed that the ethanolic extract of *Ocimum basilicum* possessed a moderate amount of anticancer activity and the IC_{50} value was recorded. The most potent anticancer activity was observed with the ethanolic extract of *O. basilicum* with IC_{50} values of 43.25µg/ml, 52.75µg/ml and 61.50µg/ml on A549, HLC1 and PC14 cells respectively. Phytochemical analyses revealed the presence of large amount of phenols and flavonoids in the potent plant extracts which may be suggested to play an important role in their anticancer activities. These results suggest that *Ocimum basilicum* is a promising source of useful natural products offers opportunities to develop the novel anticancer drugs.

***Key words*:** Anticancer, *Ocimum basilicum*, A549, HLC1 and PC14, MTT assay

A major problem of public health, cancer is one of the main causes of death globally. The prevalence of this disease is rising, however, more rapidly in Africa, Asia, and Central and South America that make up about 70% of cancer deaths in the world [1]. Cancer is one of the major human diseases and causes large suffering and economic loss world-wide. Chemotherapy is one of the methods of treating cancer. However, the chemotherapeutic drugs are highly toxic and have devastating side effects. Various new strategies are being developed to control and treat several human cancers [2]. Over 60% of anticancer drugs available in the market are of natural origin. Natural products are also the lead molecules for many of the drugs that are in use [3]. Therefore, the phytochemicals present in several herbal products and plants may have the potential to act as preventive or therapeutic agents against various human cancers [4]. The increased popularity of herbal remedies for cancer therapy perhaps can be attributed to the belief that herbal drugs provide benefit over that of allopathy medicines while being less toxic. Since the conventional therapies have devastating side effects, there is a continuous need for search of new herbal cures of cancer [5].

Many studies have been focusing on the development of agent for cancer therapies [6-7]. The chemotherapy is one of the ways to treat this disease and the advances in anticancer drugs have improved patient care. Unfortunately, the conventional chemical drugs also cause adverse side effects on normal cells/tissue, such as bone marrow function inhibition, nausea, vomiting, and alopecia [8-9]. On the other hand, natural antioxidants and many phytochemicals have been recently suggested as anti-cancer adjuvant therapies because of their anti-proliferative and pro-apoptotic properties [10]. Hence, the continuing search for anticancer agents/compounds from plants played a critical role to find the possible ways to have safe and to decrease the side effects induced by chemotherapy since natural herbal medicines have many advantages [11-13].

Apoptosis, or programmed cell death, is one of the most finely coordinated regulatory functions for maintenance of the homeostasis in the living organism. It involves the continuous checking of the cellular integrity and cascade-like events of self-destruction when the integrity of the organism is endangered. Morphological hallmarks of apoptosis are nuclear condensation, cell shrinkage, membrane blebbing and the formation of apoptotic bodies. These changes are accompanied by biochemical features, including DNA fragmentation and the proteolytic cleavage of a variety of intracellular substrates.

The Ocimum genus belongs to the Lamiaceae family [14] that includes approximately 150 species [21]. The species have variation in phenotype, oil content, composition and possibly bioactivity [15]. Although the taxonomy of basil is complicated by the existence of numerous botanical varieties

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Correspondence to: V. Ramamurthy, P. G. & Research Department of Biochemistry, Maruthupandiyar College, Thanjavur - 613 403 (Affiliated to Bharathidasan University, Tiruchirappalli), Tamil Nadu, Tel: +91 9943728616; E-mail: v.ramamoorthy07@gmail.com

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within the species that may not differ significantly in morphology, a system of standardized descriptors, which include volatile oil, has more recently been proposed by Paton and Putievsky [16]. This permits easy identification of the different forms of *O. basilicum*. Dark Opal is one of the basil cultivars that are a rich source of anthocyanin. Purple. Basils are highly marketable herbs, not only for culinary purposes but also for their ornamental value. Inter specific hybridization and polyploidy are common within Ocimum genus and purple types such as 'Dark Opal', is a possible hybrid between *O. basilicum* and *O. forskolei*, which has lobed-leaves, with a sweet basil plus clove-like aroma [17]. The anthocyanins present in purple basils have been analyzed using high performance liquid chromatography, spectral data and plasma-desorption mass spectrometry. Fourteen different anthocyanins have been identified by this analysis [18]. Apart from role of anthocyanins as pigments there are many functions performed by these flavonoid compounds in these plants (e.g., UV protection, defense against pathogens and pests, protecting DNA) and numerous scientific studies have shown that these active compounds act also as antioxidant [19]. The present investigation was taken up for evaluating the antiproliferative potential possessed by the ethanolic extract of *O. basilicum* against different lung cancer cell lines.

MATERIALS AND METHODS

Collection and Identification of plant material: For the present study, the mature plants of *Ocimum basilicum* belongs to family Lamiaceae were collected from in and around area of Thanjavur District, Tamil Nadu, South India. The plant was identified with the help of manual of Tamil Nadu and Karnatic flora [20-21] with standard references [22].

Preparation of plant materials and extract: The whole plant was shade dried and pulverized. 100gm of the powder was soaked in 150ml of ethanol (w/v) for 3-5 days with intermediate shaking. This was filtered through a fine cheese cloth and the filtrate was pooled after 3 days of repeated extractions. The filtrate obtained was evaporated to dryness using rotary evaporator. The concentrate was lyophilized and used for the study. All extracts and acyclovir (extracted from commercial tablet) were dissolved in dimethyl sulphoxide (DMSO). The final concentration of DMSO was 0.1% v/v in cell culture environment.

Phytochemical analysis: The preliminary phytochemical evaluation of leaves was carried on extract prepared by successive extraction method in Soxhlet. The resultant extracts were evaporated to dryness under vacuum. These extracts were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc. Chemical tests were identifying the phytochemicals as described [23-24]. Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Tumour cell lines: Cell lines of different tissue origin such as A549, HLC1 and PC14 were used. Cells were cultured in MEM (Minimum Essential Media) supplemented with Sodium Bicarbonate, EDTA, FCS (Foetal Calf Serum) and incubated in humidified atmosphere of 5% CO_2 and 37°C. The culture medium was changed every two days. All cell lines used were of human origin in order to more closely mimic how plant extracts would affect human cancer cells. Cells were generally cultured in 10 mL of appropriate medium in 75 cm^2 tissue culture (T-75) flasks at 37 °C in a humidified atmosphere of 5% CO_2/ 95% air. Cells were passaging weekly and medium replaced fortnightly.

MTT assay [25]: Antiproliferative effects were measured *in vitro* by using MTT ([3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide]) assays. After treatment, the living cells were assayed by the addition of 20 µl of 5 mg/ml MTT solution. Finally, the reduced MTT was assayed at 545 nm wells with untreated cells were utilized as controls. Antiproliferative and cytotoxic effects were distinguished by cell number and the duration of treatment (72 h, 5000 cells/w, and 24 h, 25000 cells/w, respectively). Stock solutions of the tested materials were prepared with dimethyl sulfoxide (DMSO). The highest DMSO concentration (0.3%) of the medium did not have any significant effect on the cell proliferation. Extracts which demonstrated potent activity (growth inhibition > 50%) were selected for further in vitro testing (dose-response curve and cytotoxicity). To study the interactions between acridones and doxorubicin, a checkerboard method was applied. A series of 2-fold dilutions of the acridones was tested in combination with 2-fold dilutions of doxorubicin. The cell growth rate was determined with MTT staining drug interactions were evaluated according to the following system (fractional inhibitory index = FIX):

FIX < 0.5	Synergism	1 < FIX < 2	Indifferent effect
FIX = 0.51-1	Additive effect	FIX > 2	Antagonism

RESULTS AND DISCUSSION

The result of phytochemical screening of the ethanolic extracts of *Ocimum basilicum* revealed that the presence of alkaloids, flavonoids, phytosterols, tannins and phenols (Table 1). The plant extract of *O. basilicum* used for the present work was choosing on the basis of their medicinal values. Previous study in the naturally the ethanolic extracts of *Ocimum basilicum* were subjected for phytochemical analysis. Phytochemical screening of the crude extract revealed that the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannin, flavonoids and steriods, but reducing sugars, carbonyl (aldehyde) and Phlobatanin show negative results [26].

These plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized by Sofowora [23]. In the present study, it was clearly understood that the ethanolic extracted maximum amount of the different type of metabolites present in the *O. basilicum* Boominathan and Ramamurthy [27] reported that the phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds.

For instance, the presence of flavonoids suggest that the plant have been reported to exert multiple biological effects including, anti-allergic, anti-inflammatory, anti- microbial antioxidant, anti- cancer activity [28]. It also suggests that the plant might have diuretic properties [29]. The presence of tannins shows that the plant is astringent as documented and suggests that it might have antiviral and anti-bacterial activities and can relief in wound healing and burns [30]. Saponins and glycoside are also very important classes of secondary metabolites as some are cardio-active and used in treatment of

heart conditions [31]. Some researchers have also investigated that some saponins have anti-cancer and immune modulatory properties [32]. Volatile oils are used in the industries for various purposes, both as a pharmaceutical/ cosmetic raw material for production of emollients and active ingredient for the respiratory tract infections.

Table 1 Qualitative phytochemical screening on extracts of *Ocimum basilicum*

S. No	Name of test	Test applied / Reagent used	Ethanol
1	Alkaloids	A] Mayer's	+++
		B] Wagner's	+++
		C] Hagner's	+++
		D] Dragndorff's test	++
2	Flavonoids	HCl and magnesium turnings	+++
3	Carbohydrate	Molisch's test	++
4	Tannins and Phenols	A] 10% Lead acetate	+++
		B] $Fecl_3$	+++
5	Test for steroids	A] Salkowski's Test	++
		B] Libermann-Burchard's Test	++
6	Gums and Mucilages	Alcoholic Precipitation	-
7	Fixed oil and Fats	Spot test	+
8	Saponins	Foam test	++
9	Phytosterols	LB test	+
10	Volatile oils	Hydro distillation method	+
11	Protein and free amino acids.	A] Biuret test	+++
		B] Ninhydrin test	+++
		C] Xanthoprotein test	+++

Fig 1 Shows the *Ocimum basilicum*

Table 2 Survival analysis of cancer cells treated with extracts of *Ocimum basilicum*

Concentrations (µg ml^{-1})	A549		HLC1		PC14	
	Cell viability (%)	Cell inhibition (%)	Cell viability (%)	Cell inhibition (%)	Cell viability (%)	Cell inhibition (%)
20	79.5	20.5	74.7	25.3	80.5	19.5
40	67.4	32.6	62.5	37.5	74.9	25.1
60	56.6	43.4	53.7	46.3	63.8	36.2
80	48.7	51.3	45.6	54.4	55.7	44.3
100	37.5	62.5	40.2	59.8	43.5	56.5
125	29.6	70.4	28.5	71.5	31.1	68.9
150	21.1	78.9	22.4	77.6	25.3	72.7
200	19.5	80.5	17.5	82.5	14.2	85.8
Vehicle control (DMSO)	100	0	100	0	100	0

Anticancer activity of *Ocimum basilicum* was studied in different mammalian cell line. Anticancer activity of ethanolic extract of *Ocimum basilicum* as well as standard was determined through MTT cytotoxicity assay. In the preliminary study, the ethanolic extract showed the good yielding capacity of phytocompounds activity. In this regard, the present investigation the ethanolic extract of *Ocimum basilicum* was studied in A549, HLC1 and PC14 cell lines and its result labelled in the (Table 2) and also made with standard drug tamoxifen. The minimum cell viability (19.5%) and maximum

cell inhibition (80.5%) were noted in 200 µg/ml concentration of *Ocimum basilicum.* The IC_{50} value (43.25µg/ml, 52.75µg/ml and 61.50µg/ml) was calculated for anticancer activity of ethanolic extract of *Ocimum basilicum* against A549, HLC1 and PC14 cell lines. The tamoxifen used as a standard for this study. In the standard, the minimum cell viability (17.5%) and maximum cell inhibition (82.5%) were observed in higher concentration. The percentage of cell inhibition was noted in the different concentrations of ethanolic extract of A549, HLC1 and PC14 ranges from 20 to 200 µg/ml.

Anticancer properties of many natural compounds isolated from different plant extracts have been reported. Research is being carried out throughout the world to find a lead compound which can block the development of cancer in humans. Nature has always been a great contributor towards this goal. Plant-derived natural products such as flavonoids, terpenoids and steroids have received considerable attention due to their diverse pharmacological properties, which include cytotoxic and chemo preventive effects [33]. They were the first agents to advance into clinical use for the treatment of cancer [34]. *Withania somnifera* as a potential source of new molecules that can curtail cancer growth were studied by Dredge *et al*. [35]. *Ocimum basilicum* has also been shown to inhibit the growth of human cancer cell lines comparable to that produced by tamoxifen. The peels extract produced antiproliferative activity on A549, HLC1 and PC14 tumor cell lines. Jayaprakasam *et al*. [36] reported that the inhibitory concentrations obtained was 25.1±0.91 against colon cell line HCT-116, but in this study plant extracts from different cancer cell treatments of *Ocimum basilicum* cultivated in fly ash containing soil had shown more than 85% inhibition against human cell lines. Furthermore, this study has reported growth inhibitory importance in *Ocimum basilicum* against various cancer cell lines i.e. A549, HLC1 and PC14 tumor cell lines. Hence, this study has revealed remarkable anticancer potential in the leaves extract of *Ocimum basilicum*.

CONCLUSION

Nowadays plants are extensively used for the research purpose and it possesses more than one chemical entity so it has been widely used for the research investigations. Anticancer properties of many natural compounds isolated from different plant extracts have been reported. Research is being carried out throughout the world to find a lead compound which can block the development of cancer in humans. Nature has always been a great contributor towards this goal. Furthermore, this study has to prove the cytotoxic effects of ethanolic extract of *Ocimum basilicum* may be conducted in clinical trials on patients suffering from cancer disease. To the best of our knowledge, the present study concluded that the *Ocimum basilicum* have an anticancer activity against A549, HLC1 and PC14 cell line. From this study, it is clear that *Ocimum basilicum* extract have significant anti-cancer activity in cell line. The anti-cancer activity is probably due to the presence of phytocompounds.

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