International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 27-08-2021, Accepted: 12-09-2021, Published: 27-09-2021
Volume 6, Issue 5, 2021, Page No. 840-845

Evaluation of *In vitro* antimitotic activity of *Ocimum basilicum* leaves extract

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Abstract
Plants are rich sources of secondary metabolites with interesting biological activities. The occurrence of cancer is due to the effect of chemicals, viruses, free radicals and some environmental and routine life factors. Traditional treatments for cancer like chemotherapy, radiotherapy and surgery provide only partial and transient relief. Also the above treatments and synthetic anticancer drugs are costly and beyond the reach of the general public. Hence, alternative herbal remedies that are commonly available and comparatively economical are to be explored. The antimitotic and antiproliferative effects are the important *In vitro* assays for the screening of anticancer compounds. In the present study to investigate the phytochemical screening and antimitotic activities of *Ocimum basilicum* leaves. The phytochemical screening *Ocimum basilicum* leaves showed that the presence of saponins, flavonoids, steroids, terpenoids, triterpenoids, anthroquinones, polyphenol, and coumarins in both extracts, significant amount of phenol (133.14mg/gm), flavonoids (25.33mg/gm) and terpenoids (10.72mg/gm). The histochemical analysis further confirmed in the presence of saponins, flavonoids, tannins, terpenoids and polyphenol. The extract of *Ocimum basilicum* leaves had excellent anti-mitotic activity. Overall, it concluded that the extract of *Ocimum basilicum* leaves has rich source of phytochemicals and anti-mitotic activity proved by *Allium* assay.

Keywords: *Ocimum basilicum*, antimitotic, anticancer, phytochemical

Introduction
Phytochemicals are also available in supplementary forms, but evidence is lacking that they provide the same health benefits as dietary phytochemicals. People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, fruits or seeds (Costa *et al*., 1999) [1]. Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. Levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions (King and Young, 1999) [2]. Mitosis or the equational division is usually restricted to the diploid cells only. However, in some lower plants and in some social insects haploid cells also divide by mitosis. It is very essential to understand the significance of this division in the life of an organism. Mitosis results in the production of diploid daughter cells with identical genetic complement usually. The growth of multicellular organisms is due to mitosis. Cell growth results in disturbing the ratio between the nucleus and the cytoplasm. It therefore becomes essential for the cell to divide to restore the nucleo-cytoplasmic ratio. A very significant contribution of mitosis is cell repair. The cells of the upper layer of the epidermis, cells of the lining of the gut, and blood cells are being constantly replaced. Mitotic divisions in the meristematic tissues – the apical and the lateral cambium, result in a continuous growth of plants throughout their life.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc (Velavan, 2011) [3]. In the present study to investigate the phytochemical screening and antimitotic activities of *Ocimum basilicum* leaves (Tamil Name: Thiruneetru pachchilai).

Materials and method
Collection of plant materials
The leaves of *Ocimum basilicum* were collected in January 2020 from Thanjavur, Tamil Nadu, India. The *Ocimum basilicum* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. Leaves were spread out in a plain paper and shade dried at room temperature for about 10 days and makes a fine

powder using grinder mixture. The powder materials were used for further studies.

Preparation for extract
One gram of the powder of *Ocimum basilicum* leaves were transferred in to different conical flask (250ml). The conical flask containing 50ml of different solution (Ethanol and aqueous). The conical flask containing *Ocimum basilicum* leaves were shake it well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using whatman filter paper No.1 and filtrate used for further analysis.

Phytochemical screening
Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993) [4], Trease and Evans (1989) [5] and Harborne (1973 and 1984) [6, 7].

Quantitative analysis of phytochemicals
Total phenols estimated by the method of Edeoga *et al*., (2005) [8]; Flavonoid determine by the method of Boham and Kocipai-Abyazan (1994) [9]; Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956) [10].

Histochemical tests: (John Peter Paul, 2014) [11].
The *Ocimum basilicum* leaves powder were treated with specific chemicals and reagents. The treated plant powder further analysed in light microscope.

Evaluation of antimitotic activity using *Allium cepa* roots
Antimitotic activity study was conducted as per the methods reported by previous workers with modifications (Grant, 1982; Fiskesjo, 1988; Shweta *et al.* 2012) [12, 13, 14].

***Allium cepa* Bulbs**
Approximately equal size bulbs (40±10 g) of the onions (*Allium cepa* L.) were obtained from the local vegetable market at Thanjavur, Tamil Nadu, India. Any onions that were dry, moldy or have started shooting green leaves were discarded.

Growing *Allium cepa* Meristems
The outer scales were removed from the healthy onion bulbs leaving the root primordia intact. These bulbs were grown in dark for 48 h over 100 ml of tap water at ambient temperature until the roots have grown to approximately 3 cm. The water was changed daily during this period. The viable bulbs were then selected and used for subsequent studies.

Exposure to Text Samples
The bulbs with root tips grown up to 2-3 cm were removed from the water and placed on a layer of tissue paper to remove excess of water. The bulbs were divided into four groups. The first group served as control (tap water). Second group: *Allium cepa* roots were dipped in the *Ocimum basilicum* leaves extract (100µg/mL). Third group: *Allium cepa* roots were dipped in the *Ocimum basilicum* leaves extract (200 µg /mL). Fourth group: *Allium cepa* roots were dipped in the *Ocimum basilicum* leaves extract (300 µg /mL). All the groups were incubated at 25±2°C for 96 h away from direct sunlight. The test samples were changed daily with fresh ones. The length of roots grown during incubation (newly appearing roots not included), root number and the mitotic index were recorded after 96 h.

Microscopic Studies and Determination of Mitotic Index
After 96 h, the root tips were fixed with fixing solution of acetic acid and alcohol (1:3). Squash preparations were made by staining the treated roots with acetocarmine stain (Badria *et al*., 2001) [15]. For each root tip, the numbers of mitotic cells and total meristematic cells were counted manually in 5-8 fields of view using high resolution (100x) bright field light microscopy. The mitotic index was calculated as

Mitotic Index = Number of dividing cells/Total number of cells x 100.

Results and discussion
Qualitative and quantitative analysis
In the present study was carried out on the *Ocimum basilicum* leaves revealed the presence of medicinally active constituents. The phytochemical characters of the *Ocimum basilicum* leaves investigated and summarized in Table 1. The phytochemical screening *Ocimum basilicum* leaves showed that the presence of saponins, flavonoids, steroids, terpenoids, triterpenoids, anthroquinones, polyphenol, and coumarins in ethanol and aqueous extracts. Tannin presence only ethanol extracts. Alkaloids and glycosides presence only aqueous extracts. Quantitative analysis revealed that the *Ocimum basilicum* leaves powder has significant amount of phenol (133.14mg/gm), flavonoids (25.33mg/gm) and terpenoids (10.72mg/gm) were presented (Table 2). The above phytoconstituents were tested as per the standard methods.
Hassain *et al.* (2011) [16] screened phytochemical constituents from methanol leaf extract of *Bombax malabaricum*. Various organic 11 solvent extracts of *Pedalium murex* were subjected to preliminary phytochemical screenings by Thamizh mozhi *et al.* (2011) [17]. Selected 53 traditionally used medicinal plants from western region of India for their qualitative phytochemical screenings, total phenol and flavonoids contents. Pascaline *et al.* (2011) [18] screened phytochemical constituents of some medicinal plants used by the Nandis of South Nandi District, Kenya.
Kumar *et al*., (2013) [19] investigated the preliminary phytochemical screening of the leaves of the plant *Lasia spinosa* (Lour) Thwaites. The phytochemical screening showed that the methanol and aqueous extracts contained alkaloid, the carbohydrates and the phenolic compounds were present in all of the solvent extract except petroleum ether extract. The chloroform, ethyl acetate and the aqueous extract contained glycosides whereas the saponins present in methanol and aqueous extract. The ethyl acetate extract contain only the flavonoids.

Table 1: Qualitative analysis of Phytochemicals in *Ocimum basilicum* leaves extract

S. No	Phytochemicals	Extract	
		Aqueous	Ethanol
1	Tannin	-	+
2	Saponin	+	+
3	Flavonoids	+	++
4	Steroids	+	+
5	Terpenoids	+	+
6	Triterpenoids	+	+
7	Alkaloids	+	-
8	Antroquinone	++	+
9	Polyphenol	+	+
10	Glycoside	+	-
11	Coumarins	+	+

(+) Presence, (++) High concentrations and (–) Absences

Table 2: Quantitative analysis of Phytochemicals in *Ocimum basilicum* leaves powder

Phytochemicals	Results (mg/ml)
Poly phenol	133.14 ± 9.32
Flavonoids	25.33 ± 1.77
Terpenoids	10.72 ± 0.72

Values are expressed as mean ± SD for triplicates

Leo Stanley *et al.* (2011) [20] reported that leaves of *C. pedata* showed the presence of alkaloids, carbohydrates, steroids, tannin, phenolic compounds, flavonoids and terpenoids. Dinesh kumar *et al.* (2011) [21] has been reported to terpenoids, flavonoids and tannin are present in *C. trifolia*. Rajmohanan *et al.* (2014) [22] investigated the preliminary phytochemical analysis of various extracts of leaves of *C. pedata* and showed the presence of carbohydrates, flavonoids, tannins and phenolic compounds and terpenes.

Histochemical analysis of *Ocimum basilicum* leaves powder

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues, it is a powerful tool for localization of trace quantities of substances present in biological tissues. Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major phytocompounds (Krishnan *et al.*, 2001) [23]. In the present study, *Ocimum basilicum* leaves powder was treated with specific chemicals and reagents. The *Ocimum basilicum* leaves powder treated with diluted ammonia and H_2SO_4 gave yellow colour indicates Flavonoids, treated with Few drops toluidine blue reagent gave Blue green / red colour indicates Polyphenol, treated with Few drops Con. H_2SO_4 reagent gave Yellow colour indicates Saponins and treated with Few drops Dinitrophenol hydrazine reagent gave Orange colour indicates Terpenoids (Table 3 plate 1**)**. This results further confirmed the presence of phytochemicals.

Table 3: Histochemical analysis of *Ocimum basilicum* leaves powder

S. No	Phytochemicals	Colour Observation	Results
1	Flavonoids	Yellow	+
2	Saponins	Yellow	+
3	Tannin	Blue to black	++
4	Polyphenol	Blue	++
5	Terpenoids	Orange	++

Note: (+) Presence; (++) present with high intensity of the colour

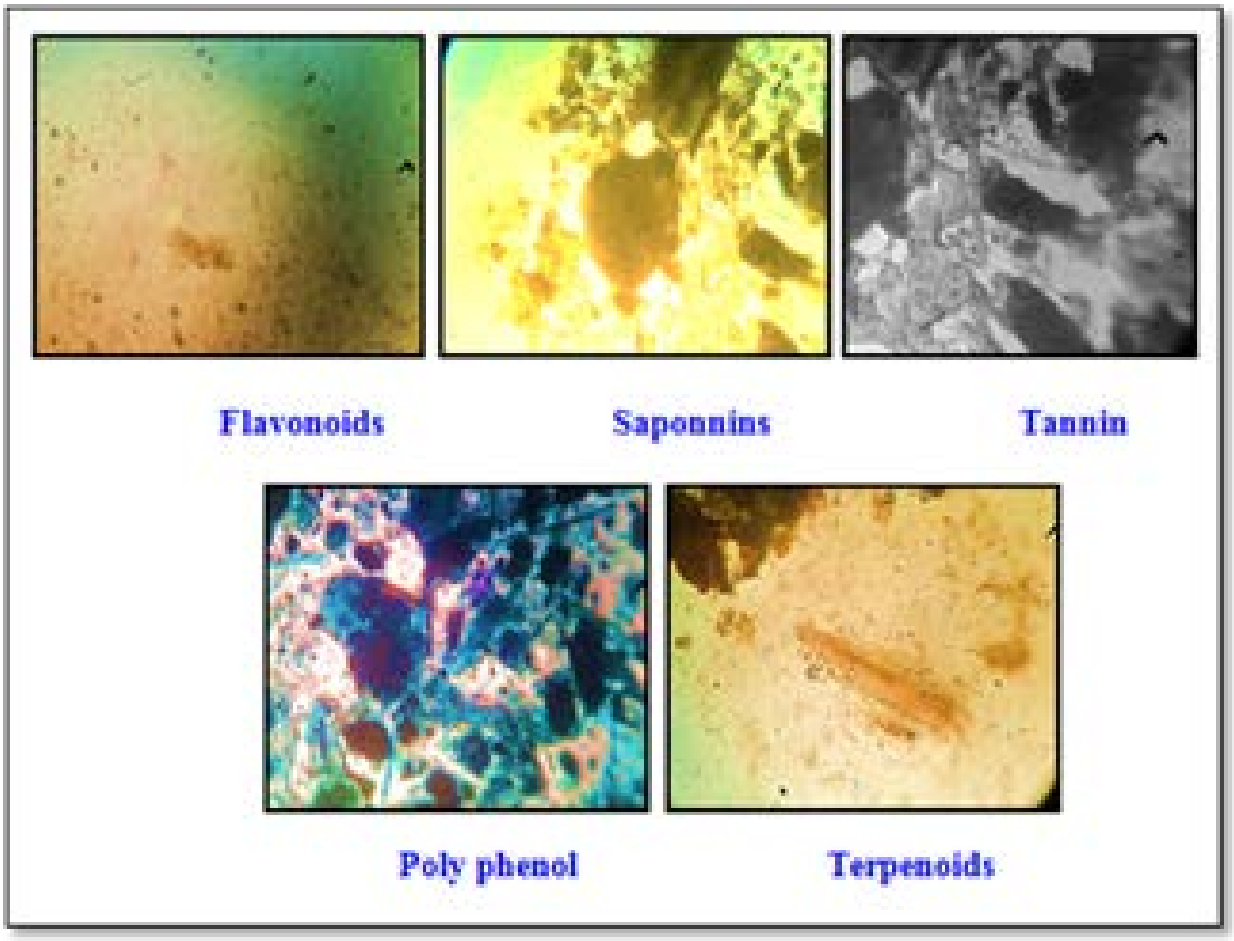


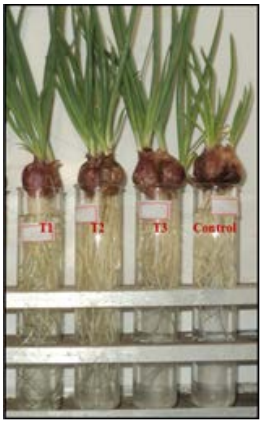
Plate 1: Histochemical analysis of *Ocimum basilicum* leaves powder

Antimitotic activity of *Ocimum basilicum*

In *Allium cepa L*. root tip model root system of plant cells is commonly used as a test for investigating environmental pollution factors, toxicity of chemical compounds and evaluating potential anticancer properties. It is very comfortable as it is easy to make preparations of onion roots. They contain rather homogenous meristematic cells, having only 16 chromosomes, which are very long, well visible and get stained easily. The test is a fast and inexpensive method, allowing the investigation of universal

mechanisms for meristematic plant cells and extrapolation on animal cells (Kuras *et al*. 2006) [24]. The aim of this work was investigating the antimitotic activity of *Ocimum basilicum* leaves.

The methanolic extract of *Ocimum basilicum* leaves produced root decay and decreased the root length and root number significantly at 96 h as compared to control ($p<0.05$). The average root length in control, 100, 200 and 300µg/mL of *Ocimum basilicum* leaves was 0.50, 0.10, 0.20 and 0.30mm at 96 hr respectively. The root numbers in control 100, 200 and 300µg/mL of *Ocimum basilicum* leaves was 10, 13, 21 and 5 at 96 hr respectively. The mitotic index at 100, 200 and 300µg/mL of *Ocimum basilicum* leaves was 45.80, 35.91 and 32.12% at 96 hr respectively. The highest dose as 30mg/mL of *Ocimum basilicum* leaves has significant activity in root length, number and mitotic index and near to the standard (Table 4, 5, Figure 1, 2 and Plate 2, 3).



T1 (100 µg/ml), T2 (200 µg/ml), T3 (300 µg/ml) and Control (water)

Plate-2: Treatment of *Ocimum basilicum* extract in *Allium cepa* roots

Table-4: Effect of *Ocimum basilicum* on root length, root number and mitotic index of *Allium cepa* roots

Groups	Mean root length (mm)				Mean root Number (s)		
	Before treatment	After treatment	Average root growth	% of root growth inhibition	Before Treatment	After Treatment	Average root number
Group I (Water control)	12.00	12.50	0.50	-	42	52	10
O. basilicum (100µg/ml)	11.50	11.60	0.10	20	47	60	13
O. basilicum (200µg/ml)	16.50	16.70	0.20	40	45	66	21
O. basilicum (300µg/ml)	14.20	14.50	0.30	60	68	73	5

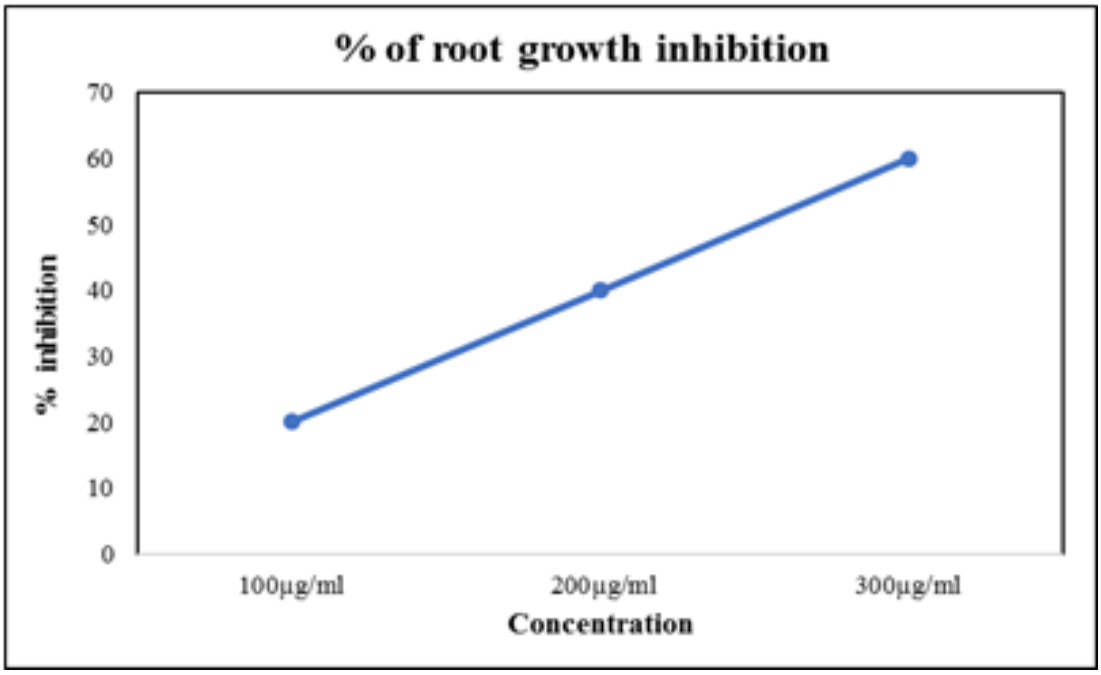


Fig 1: Effect of *Ocimum basilicum* on % of root growth inhibition of *Allium cepa* roots.

Table-5: Effect of sample on mitotic index of *Allium cepa* roots

Groups	Dividing cells	Non dividing cells	Total number of cells	Mitotic index (%)
Group I (Water control)	45	30	75	60
O. basilicum (100µg/ml)	142	168	310	45.80
O. basilicum (200µg/ml)	130	232	362	35.91
O. basilicum (300µg/ml)	124	261	386	32.12

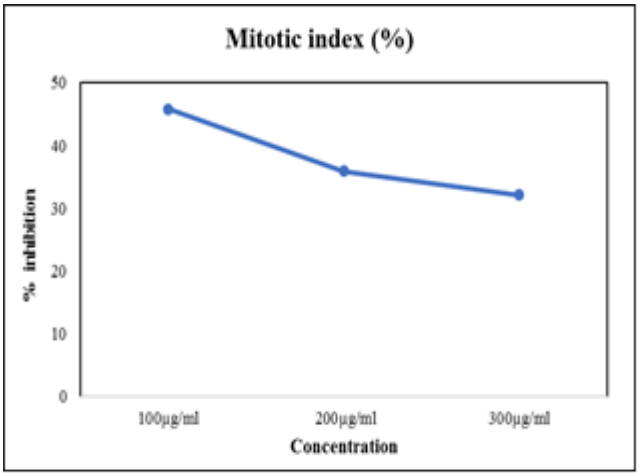


Fig 2: Effect of *Ocimum basilicum* on mitotic index of *Allium cepa* roots

Morphometric study on *Allium cepa* roots with extract of *Ocimum basilicum*

The water control shows normal growth with greater root length and numbers. Treatment with different concentrations (100, 200 and 300µg/mL) of *Ocimum basilicum* extract shows decreased the growth gradually in dose dependent manner. The highest dose (300µg/mL) and standard has significantly reduced the root length and number compared to other doses and near to the standard (Plate 3).

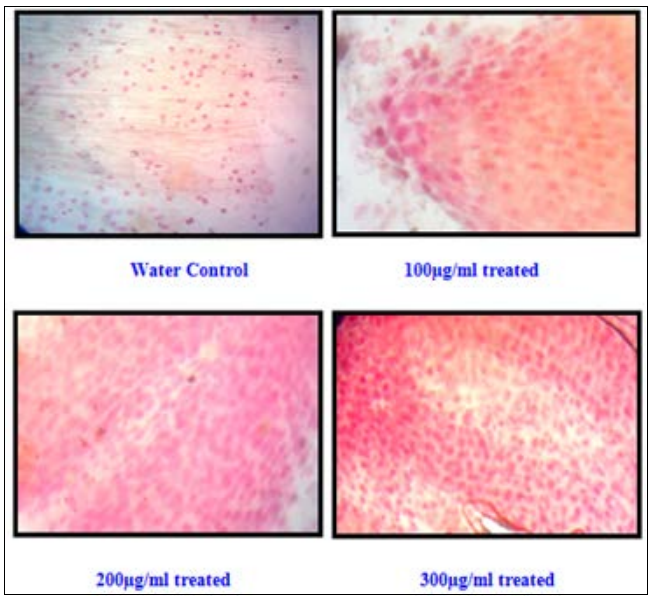


Plate 3: Photomicrograph treatment of *Datura stramonium* extract on mitotic index in *Allium cepa* roots

The antimitotic activity was screened using *Allium cepa* root meristamatic cells which have been used extensively in screening of drugs with anticancer activity (Abhang *et al*., 1991; Latha *et al*., 1998) [25, 26] The roots of all plants have distinguished regions, one of them being the region of cell division that lies beyond the root cap and extends a few mm after that. Cells of this region undergo repeated divisions. The fate of cell division is higher in this region compared to that of the other tissues. This region is called the meristamatic region (meristos: divided) (Dutta, 1971) [27].

Conclusion

Overall, it concluded that the above results suggest that the extract of *Ocimum basilicum* leaves has rich source of phytochemicals confirmed by qualitative and quantitatively. The biological activity as antioxidant evidenced by anti-mitotic activity proved by *Allium* assay. The present investigation provides comprehensive *In vitro* that anti-mitotic demonstrates remarkable cytotoxic properties thus suggesting the feasibility of its possible promise as natural antitumor agent.

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