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PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTICANCER POTENTIAL OF *IMPERATA CYLINDRICA* (L.) RAEUSCH AGAINST HUMAN BREAST CANCER CELL LINE (MCF-7)

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ABSTRACT

The traditional knowledge on plants when confirmed by phytochemical, antioxidant and anticancer studies can lead to the development of drugs and plant-based medicine. The plant selected for the present study Imperata cylindrica is a rhizomatous grass used for various ailments by traditional healers. The present study aims at phytochemical profiling of whole plant extracts of I. cylindrica and to demonstrate its efficacy as an antioxidant and anticancer agent against human breast cancer cell line (MCF-7). Methanol, ethanol, petroleumether, chloroform and aqueous whole plant extracts of I. cylindrica was screened to identify the bioactive compounds and to evaluate its bioefficacy. Phytochemical (qualitative and quantitative) screening of the whole plant extracts of I. cylindrica revealed the presence of bioactive compounds as tannins, saponins, flavonoids, alkaloids, quinines, glycosides, terpenoids, phenols, coumarin and steroids in all the solvent extracts. Fifty percent inhibition of free radical scavenging activity was observed only in the methanolic extract of I. cylindrica at 59.74µg/ml when compared to the other extracts. The cytotoxicity was evaluated using the changes in cell morphology, cell viability and nuclear staining and the percentage of cell viability was determined by MTT assay. Our results showed that methanolic extract of I. cylindrica inhibited the proliferation of human breast cancer cell line MCF-7 with an IC_{50}value of 83.10µg/ml at 24 h incubation, and was shown to promote apoptosis as seen inpropidium iodide staining. These results suggest that methanolic extract of I.cylindrica has antiproliferative effect against MCF-7 cell by suppressing its growth.

KEY WORDS

Imperata cylindrica, phytochemical, antioxidant, anticancer potential.

INTRODUCTION

India has a rich flora that is widely distributed throughout the country. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Several plant species are used by many ethnic groups for the treatment of various ailments ranging from minor infections to dysentery, skin diseases, asthma, malaria and a horde of other indications [1-4]. Population rise, inadequate supply of drugs, prohibitive cost of treatment, side effects of several allopathic drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicine for a wide variety of human ailments [5]. Natural products from plants have also been valuable sources of anticancer drug discovery [6].

The plant for the present study *Imperata cylindrica* belongs to the family Poaceae known as Thatch grass or Cogon grass (English), Darbh (Hindi) [7, 8] and Dharbai pullu (Tamil) [9]. It is a perennial, monocot plant. It is an important drug of Tripanchmool and used in urinary

calculi, retention of urine, diabetes, cardiac disorders, gout, common cough and cold, anaemia [7].

Cogongrass is one of the 10 most troublesome and problematic weed species in the world. It is a perennial, rhizomatous grass endemic to tropical and subtropical regions throughout the world and often overtakes areas disturbed by human activities [10]. In Asia Cogongrass is considered as a medicinal plant, and the rhizome is used for medicinal purpose [11].

The edible part of the plant includes young inflorescence and young shoots which are cooked and fibrous root contain starch and sugar which is pleasant to chew. Apart from edible use, the medicinal uses include antibacterial, anthelmintic [12], astringent, anti-inflammatory [13], antihypertensive [14] etc. It is effective in conditions like arthritis, diarrhoea, dysentery, gonnorhea, diuretic, emollient [15]. These plants are also used for soil stabilization, stuffing, thatching, and paper industry [16] and also in weaving [17].

Even though there are various reports on the biological activity of *I.cylindrica* there is a paucity of data on their antioxidant and anticancer properties. The present study analysed the phytochemicals present through qualitative and quantitative phytochemical screening methods. It also investigated the antioxidant activity of the whole plant extracts of *I.cylindrica* and its anticancer potential against MCF-7 cell line.

MATERIALS AND METHODS

Collection of plant samples

Healthy, disease free plants of *I. cylindrica* were collected from the fields in Nathammedu village in Tiruvallur district (near Chennai). The plant material was identified and authenticated based on the morphology characteristics by Prof.P.Jayaraman, Plant Anatomy Research Center (PARC), Tambaram, Tamil Nadu, India. Washed and air-dried plant were cut into small pieces and pulverized in a domestic blender and used for the preparation of aqueous and solvent extracts.

Preparation of extracts

I. cylindrica were collected and dried under shade at room temperature for about 20 days. The dried plant was powdered and sieved to get fine powder using an electric blender. 50 g of air-dried powder was then extracted with 100 ml of solvents *viz*., petroleum ether, chloroform, methanol, ethanol and aqueous. The sample was kept in dark for 72 h with intermittent shaking. After incubation, the solution was filtered through Whatman filter paper No. 1 and the filtrate was collected (crude extracts) and were stored in an air tight container for the further biological study.

Phytochemicals screening

The phytochemical screening was carried out using standard procedure [18]. By this analysis, the presences of several phytochemicals listed in (Table 1) were tested.

Table 1: Qualitative phytochemical screening of whole plant extracts of *I. cylindrica*

S.No	Phytochemicals	Aqueous	Methanol	Ethanol	Petroleum ether	Chloroform
1	Carbohydrates	+++	+++	+++	+++	+++
2	Tannins	-	+++	-	+++	-
3	Saponins	+++	++	+	-	+
4	Flavonoids	++	++	++	+	+++
5	Alkaloids	+	+++	+	+++	+
6	Anthocyanin	-	+++	+++	+++	+++
7	Quinones	-	++	+	-	-
8	Glycosides	-	+	+	-	+
9	Cardio Glycosides	+++	+++	++	-	-
10	Terpenoids	+++	++	++	-	+
11	Triterpenoids	-	-	-	-	-
12	Phenols	+++	+++	+++	+	++
13	Coumarins	+	+++	+++	+	+++
14	Acids	-	++	++	-	++
15	Protein	-	-	-	-	-
16	Steroids	+++	++	-	-	-

+++ Strongly positive, ++ Positive, + Trace, - Not detected

Table 2: Quantitative analysis of phytochemical constituent of methanol extract of *I. cylindrica*

S.No	Phytochemicals	mg/gm of extract
1.	Tannins	2.54
2.	Saponins	0.411
3.	Flavonoids	2.34 (Rutin)
4.	Alkaloids	10.65
5.	Quinone	2.43
6.	Glycosides	24.3
7.	Terpenoids	2.53
8.	Phenols	1.94 (Gallic acid 0.84, Ellagic acid 0.38)
9.	Coumarin	0.835
10.	Steroids	2.49

Table 3: DPPH radical scavenging activity of whole plant extracts of *I. cylindrica*

S.No	Concentration of extract (µg/ml)	% Inhibition*					
		Petroleum ether	Chloroform	Methanol	Ethanol	Aqueous	Ascorbic acid
1.	20	1.92 ± 0.004	2.25 ± 0.003	32.67 ± 0.004	7.82 ± 0.004	1.66 ± 0.003	31.74
2.	40	11.07 ± 0.004	4.31 ± 0.002	37.57 ± 0.002	20.01 ± 0.003	15.57 ± 0.004	40.95
3.	60	23.72 ± 0.003	8.08 ± 0.005	50.17 ± 0.004	27.77 ± 0.001	25.98 ± 0.006	53.55
4.	80	29.42 ± 0.007	16.1 ± 0.002	68.99 ± 0.004	37.51 ± 0.003	33.33 ± 0.006	62.96
5.	100	38.7 ± 0.003	26.44 ± 0.0	77.27 ±0.004	52.62 ± 0.002	47.18 ± 0.004	78.00

*Mean ± SD of three determinations.

Table 4: Exact IC_{50} value of *I. cylindrica* by DPPH free radical scavenging activity

Extract / Standard	IC_{50} value
Petroleum ether	124.36
Chloroform	145.58
Methanol	59.74
Ethanol	96.54
Aqueous	104.07
Ascorbic acid	54.34

Table 5: Per cent cell viability of MCF-7 cells for 24 hours when treated with methanolic extract of *I. cylindrica*

Concentration (µg/ml)	% Cell viability*	IC_{50}=83.10 µg/ml
Control (0)	100	
25	89.016 ± 0.605045 (-10.98)	
50	77.898 ± 0.275445 (-22.10)	
75	63.328 ± 0.269574 (-36.67)	
100	51.288 ± 0.376191 (-48.71)	
125	26.808 ± 0.725927 (-73.19)	

*Mean± SD of three determinations. Values in bracket represent % change.

Antioxidant potential of whole plant extracts of *I. cylindrica* by DPPH assay

The free radical scavenging activity of purified extract was measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) according to the method of Zhang *et al*. [19]. A total of 2 ml of DPPH (0.1 m mol/l) solution in methanol was added to different concentrations of purified extract (20–100 µg/ml), shaken vigorously, allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm. A low absorbance of the reaction

mixture indicated a high free radical scavenging activity. Sample blank and positive control was performed according to the method. Scavenging effect of DPPH radical was calculated using the following equation:

DPPH radical scavenging activity (%) =

[1-(A sample - A sample blank/A Control) ×100]

Where A sample is the absorbance of DPPH solution and test sample, A sample blank is the absorbance of the sample only without DPPH solution. Synthetic antioxidant ascorbic acid was used as positive control.

Human breast adenocarcinoma (MCF-7) cell line

Human breast adenocarcinoma (MCF-7) **c**ell line used for the present study was procured from National Centre for Cell Science (NCCS), Pune, India.

Preparation of growth medium

Ten grams of Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich St. Louis, USA) was dissolved in 990 ml of sterilized double distilled water. To this solution, 1.5 g of sodium bicarbonate and 10 ml of gentamycin cocktail were added and mixed thoroughly. Later this medium was filtered using membrane filter (0.22 µm), dispensed into sterilized container and stored at 4°C. Fetal Bovine Serum (FBS) (10%) was added to this medium and used for cell culture.

Cell line and passage

The cells were grown in T-75 culture flask containing DMEM supplemented with 10% FBS and the flask was placed at 37°C in humidified incubator with 5% CO_2. When the cells reached 70-80% confluent, the spent medium was discarded and the monolayer was rinsed with Phosphate Buffered Saline (PBS). Trypsin-EDTA solution was added and placed in incubator for 2 min. After incubation, 5.0 ml of growth medium was added to the flask and mixed gently. Then it was transferred into a 15 ml falcon tube and centrifuged at 1000 rpm for 5 min. The supernatant was carefully aspirated and the pellet was gently resuspended in 2.0 ml of growth medium. The cells were diluted with appropriate volume of growth medium and the aliquot was transferred to a new culture flask at the density of $2\times10^3/cm^2$ and kept back to controlled environment for large scale production.

Standardization of crude extracts

Standardization of crude extracts against human breast adenocarcinoma (MCF-7) **c**ell line was done by MTT assay.

Cell viability assay (MTT Assay)

Cell viability was assessed by the MTT method as described by Mosmann [20]. MCF-7 cells (1×10^4 cells/ml) were plated in 96 well plates with DMEM containing 10% FBS. The cells were incubated for 24 h under 5% CO_2 and 95% O_2 at 37°C. The medium was removed, washed with PBS and fresh serum free medium was added and kept in incubator for 1 h. After starvation, the cells were treated with the methanolic extracts of *I.cylindrica* at different concentrations such as 25, 50, 75, 100 and 125µg/ml and incubated for 24 and 48 hrs. After incubation, 10 µl of 5.0 mg/ml MTT solution was added to each well and incubated for 4 h. After incubation, supernatant was aspirated and 100 µl of DMSO was added to solubilize the crystals. A micro plate reader was used to measure the absorbance at 570 nm for each well. Percentage of cell viability was calculated as follows:

$$\textbf{Cell viability (\%)} = \frac{\textbf{OD of Experimental Sample}}{\textbf{OD of Control Sample}} \times 100$$

Cell morphological study

The morphological changes of *I. cylindrica methanolic* extract treated MCF-7 cell lines were assessed by using light microscopy. Cancer cells (1×10^6 cells/ml) were plated in 100 mm dishes and incubated for 24 h under controlled environment. Then, the spent medium was removed, followed by addition of fresh medium with or without methanolic extract of *I. cylindrica* at an inhibitory concentration and incubated for 24 h. After incubation, the cells were visualized under radical inverted light microscope at 20X magnification.

Propidium iodide staining

The treated MCF-7 cells were plated at $5x10^4$ cells/well into a six well chamber plate. At >90% confluence, the cells were treated with methanolic extract for 24 h. The cells were washed with PBS, fixed in methanol: acetic acid (3:1 v/v) for 10 min and stained with 50 mg/ml propidium iodide for 20 min. Nuclear morphology of apoptotic cells with condensed/fragmented nuclei was examined under confocal microscope [21].

RESULTS AND DISCUSSION

Qualitative and quantitative phytochemical analysis

The preliminary phytochemical screening of the whole plant extracts of *I. cylindrica* (Tables1 and 2) revealed the presence of various chemical compounds such as tannins, saponins, flavonoids,alkaloids, anthocyanin, quinines, glycosides, terpenoids, phenols, coumarins

and carbohydrates. Quantitative phytochemical analysis of methanol extract revealed the presence of rutin (2.34mg), gallic acid (1.94 mg) and ellagic acid (0.38 mg). Since there are no reportsavailable exclusively on whole plant of *I.cylindrica*. The phytochemical content in the present study was comparable with the available literature on phytochemical screening of root extract of *I.cylindrica* [12]. The chemical constituents in the rhizome of *I.cylindrica* were reported by Liu Rong-hua*et al*.[22] and Li Li-shun *et al*. [23].

Antioxidant potential by DPPH assay

Fifty percent inhibition of free radical scavenging activity was observed only in the methanolic extract of *I. cylindrica* at 59.74µg/ml when compared to the other extracts of *I. cylindrica*. Finally, methanolic extract also showed similar free radical scavenging activity whencompared to the positive control ascorbic acid as shown in Tables3 and 4, (Figure 1). Similar free radical scavenging activity was reported by Padma *et al*. [24] in the methanolic root extract of *I. cylindrica.* In a study by Xian-rong Zhou *et al*. [25] also reported a good anti-oxidation ability of extracts of the rhizomes of *I. cylindrica.*

Figure 1: DPPH radical scavenging activity of whole plant extracts of Imperata cylindrica

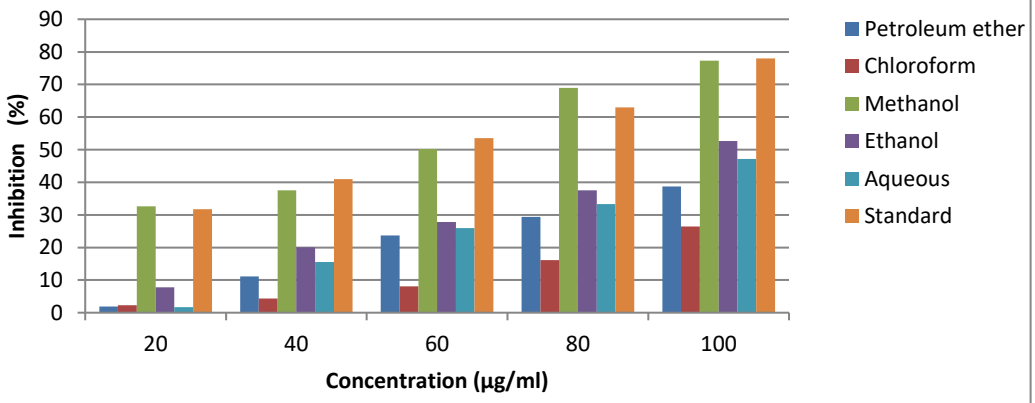


Figure 2: Line diagram showing decrease in viability of MCF-7 cells treated with methanolic extract of *I. cylindrica* for 24 h

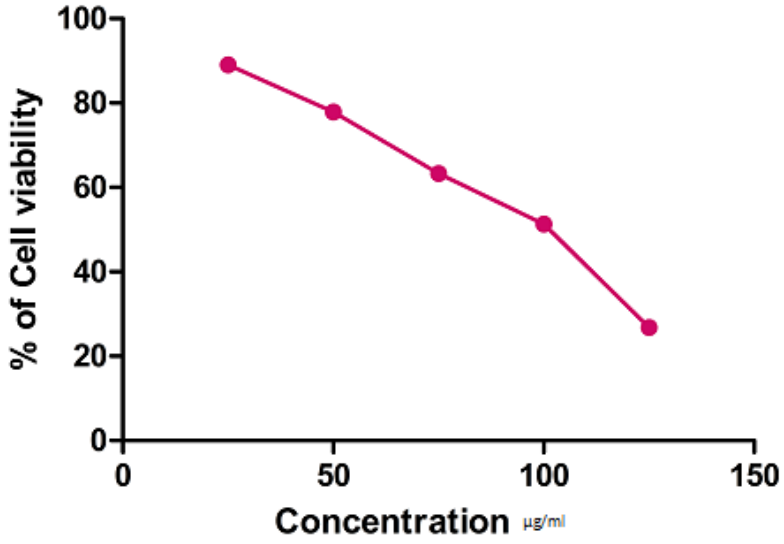


Figure 3: Morphological changes of MCF-7 cells induced by methanolic extract of *I. cylindrica*

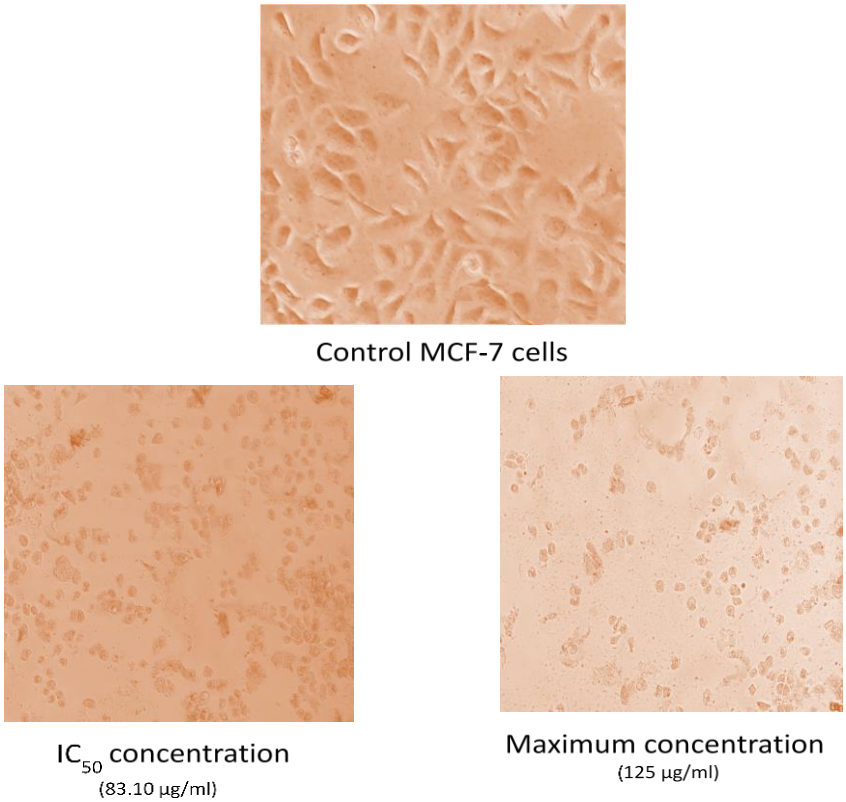
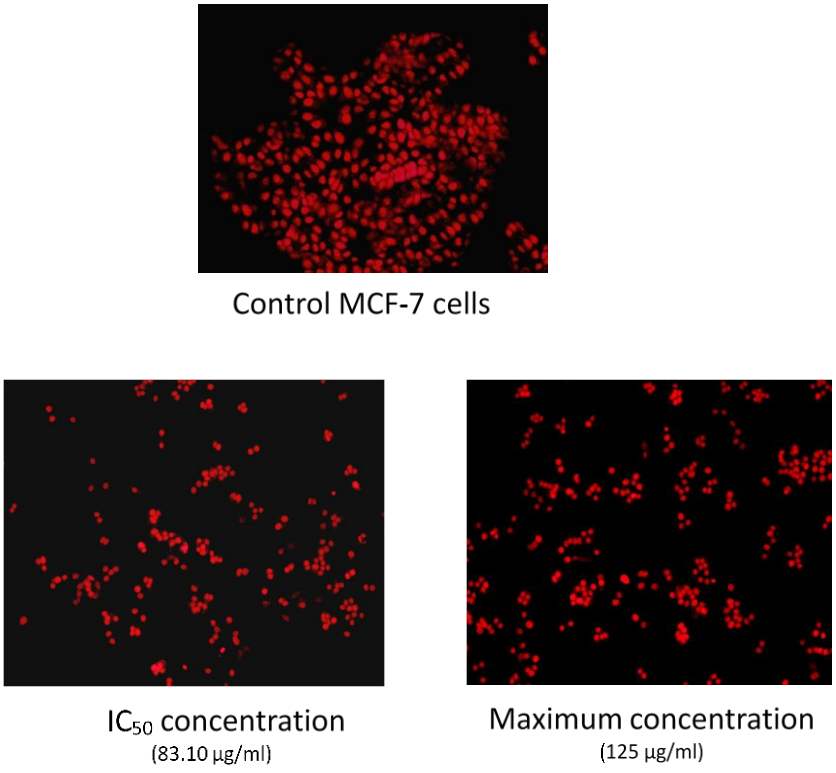


Figure 4: Nuclear morphology of apoptotic cells when treated with methanolic extract of *I. cylindrica*



Free radicals called reactive oxygen species (ROS) are normal products of human metabolism. The human body has evolved a mechanism to neutralize the oxidative stress by producing antioxidants naturally *in situ* such as catalase, superoxide dismutase, and glutathione peroxidase. These antioxidant enzymes may be supplied externally through foods and supplements [26]. However, antioxidant defence mechanism does not meet demand in the case of increase of ROS level in several diseases. Damage on the tissues occurs by isoprostanes generated by lipid peroxidation, increase in the content of aldehydes, or protein carbonyls produced from protein oxidation and oxidized base adducts generated from DNA oxidation [27]. Nowadays, a large number of studies have reported the antioxidant activities of plant-based substances, thus resulting in evolution of herbal drugs in complementary and alternative folkloric medicine in the world.

Anticancer activity (MTT assay)

Cell viability was assessed using anticancer activity by MTT assay for 24 hours. The anticancer activity was increased with increase in concentration. The anticancer activity was noted in methanolic extract of *I. cylindrica*, which was directly proportional to the concentration of the methanolic extract. The inhibitory concentration (IC_{50}) was obtained at 83.10µg/ml as shown in Table 5 and Figure 2. The morphological observation of MCF-7 cancer cells was photographed in Figure 3. The control showed irregular confluent aggregates with polygonal cell morphology. The cell shrinkage increased progressively and it was done and time dependent. Manosroi *et al*. [28] reported in their study that a recipe containing *I. cylindrica* as one of its constituents gave the high anti-proliferation activity on KB cell lines. Keshava *et al*. [29] were the first to report the anticancer activity of the methanol extracts from the leaves of *I. cylindrica* in human oral squamous carcinoma cell line SCC-9 *in vitro*.

Propidium iodide staining

To confirm whether the cytotoxic effect induced by methanolic leaf extract of *I. cylindrica* involves apoptotic changes, the nuclear condensation was studied by the propidium iodide staining method. In the case of control cells, a variety negligible number of propidium iodide positive cells were present. In the case of cells treated with 83.10µg/ml of methanolic leaf extract for 24 hours, a progressive increase in the number of propidium iodide positive cells was observed (Figure 4). Apoptosis is characterized by distinct morphological features such as cell shrinkage, chromatin condensation, plasma membrane blebbing, oligonucleosomal DNA fragmentation and finally breakdown of the cell into smaller units (apoptotic bodies).

CONCLUSION

The continuous emergence of diseases, the emergence of drug resistant organisms and increasing prices of medicines call for discovery of new less expensive plant-based medicines. The qualitative and quantitative phytochemical analysis revealed that the solvent extract of *I. cylindrica* contains carbohydrates, tannins, saponins, flavonoids, alkaloids, anthocyanin, glycocides, cardioglycocides, phenols, coumarins and steroids. In addition, the methanolic extract of *I. cylindrica* exhibited significant free radical scavenging and antioxidant activity *in vitro*. Moreover, it also showed significant anticancer property against human breast cancer cell line (MCF-7). Further studies are needed for isolation, structural elucidation, and screening of any of the above-mentioned active principles to propose the activity of a drug. This study provides experimental evidence and supports the folkloric use of this plant and lends pharmacological credence to the ethnomedical use in traditional system of medicine. Also, this study demands further studies to elaborate its use, active constituents and safety.

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