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Research Article

Evaluation Of Cytotoxicity Of Root Sample Of Shankapushpi (Clitoria Ternatea) In Breast Cancer Cells (MCF-7)

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ABSTRACT

Breast cancer remains a leading cause of cancer-related mortality worldwide, necessitating the exploration of novel therapeutic agents. The search for effective anti-cancer agents from natural sources is an ongoing endeavor in cancer research. This study aimed to evaluate the anti-cancerous properties of the root extract of Shankapushpi (*Clitoria ternatea*), a traditional Ayurvedic herb renowned for its cognitive-enhancing, anxiolytic, and neuroprotective properties on human breast cancer cells. We investigated the cytotoxic effects of the root extract on MCF-7 breast cancer cell lines using various in vitro assays. Contrary to our expectations, the results indicated that Shankapushpi root extract did not exhibit significant cytotoxicity or inhibit the proliferation of breast cancer cells. These findings suggest that the root of Shankapushpi lacks efficacy in inhibiting breast cancer cell growth under the conditions tested. Further research is necessary to explore other parts of the plant or different extraction methods that might yield more promising anti-cancer properties.

Keywords: Breast Cancer, Anti Cancerous Properties, *Clitoria ternatea*, Cytotoxic Effects, Root Extract

Introduction

Cancer is a life-threatening condition and remains one of humanity's greatest health challenges, necessitating an effective treatment strategy. Breast cancer, with 2.26 million cases worldwide, is the most commonly diagnosed cancer and is the fifth leading cause of cancer-related deaths in women. Despite advancements, various therapies face significant drawbacks that hinder clinical treatment success. The primary cancer treatments—surgery, chemotherapy, and radiotherapy—are often complicated by issues such as metastasis, drug resistance, toxicity, and undesirable side effects. Additionally, cancer relapse is a frequent problem due to the persistence of malignant cells and cancer stem cells. The persistent challenges in cancer therapy are exacerbated by the resistance of cancer cells to synthetic drugs. Consequently, there is a continuous search for more effective methods of chemoprevention and chemotherapy. In this context, phytochemicals have attracted increasing attention for their potential role in cancer prevention and treatment.[1,2,3]
Breast cancer is the most prevalent type of cancer among women globally. Among the various types of breast cancer, triple-negative breast cancer (TNBC) is particularly aggressive, challenging to treat, and has a high likelihood of metastasis in diagnosed patients. Women with TNBC face a poor prognosis and limited treatment options, highlighting the critical need for new therapeutic agents.[4,5] Many studies indicates that flavonoids can inhibit cancer cell proliferation and delay tumor progression by suppressing metastasis and angiogenesis and by regulating apoptosis-related signaling pathways such as the Akt and PTEN pathways. Consequently, consuming foods rich in flavonoids may aid in preventing the initiation or early progression of cancer cells.[6]
Clitoria ternatea, also known as Asian pigeonwings, bluebellvine, blue pea, butterfly pea, cordofan pea, or Darwin pea, is a plant species from the Fabaceae family, native to the Indonesian island of Ternate. It is a perennial vine or creeper with elliptic, obtuse leaves and thrives in moist, neutral soil. The plant is renowned

for its vibrant deep blue flowers with light yellow markings, measuring about 4 cm long and 3 cm wide. Some varieties produce white or pink flowers.

The plant's fruits are flat pods, 5–7 cm long, containing six to ten seeds each, which are edible when young. *Clitoria ternatea* is commonly cultivated as an ornamental plant and for land rehabilitation purposes, such as in coal mines in Australia, due to its low maintenance needs. As a legume, it forms a symbiotic relationship with soil bacteria called rhizobia, which convert atmospheric nitrogen into a usable form for plants, thus enhancing soil quality through nitrogen-rich plant decomposition. Chemical compounds from *C. ternatea* include triterpenoids, flavonol glycosides, anthocyanins, and steroids. Notably, the plant contains cyclic peptides called cliotides and anthocyanins, particularly ternatins, which give the flowers their distinctive blue color. [7,8,9,10] In the current study, we evaluated the anticancer effects of shankapushpi on breast cancer cells by assessing its cytotoxicity.

Materials and Methods

Plant sample preparation

Roots of *Clitoria ternatea* were washed properly and sundried until it lost its complete moisture. After drying it was ground to fine powder using pestle and mortar and seived.

Fig 1: Root Samples of *Clitoria ternatea*

Cell culture

MCF-7 cell lines were procured from ATCC, stock cells were cultured in DMEM with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100µg/mL) in a humidified atmosphere of 5% CO2 at 37°C until confluent. The cells were dissociated using 0.05% trypsin and centrifuged at 1000 rpm for 5 mins. The culture media was discarded and the cell pellet was gently re-suspended using 2 ml DMEM complete media. The viability of the cells was checked and a single cell suspension of 5.0 x 105 cells/ml was prepared. Source of reagents: DMEM, FBS, Penstrep, Trypsin-procured from Invitrogen.

Table 1: Reagents

Si. No	Particulars	Source	Catalogue No
1	T25 flask	Falcon	353108
2	DMEM Medium	Gibco	31600034
4	Trypsin EDTA 0.05%	Gibco	25300062
5	FBS	Gibco	10082147
8	MTT reagent	Sigma	M5655
9	DMSO	Sigma-Aldrich	D2650

Preparation of test solutions

Standard: Doxorubicin

10 mM stock of Doxorubicin was taken. Further Serial two fold dilutions were prepared from 100 µM to 3.125 µM using) plain media for treatment. (Media used for particular cell lines mentioned in procedure)

10 mM
(30 µl of 10 mM + 270 µl of plain media)
1 mM
(30 µl of 1 mM + 270µl of plain media)
100 µM
(150 µl of 100 µM + 150 µl of plain media)
50 µM
(150 µl of 50 µM + 150 µl of plain media)
25 µM
(150 µl of 25 µM + 150 µl of plain media)
12.5 µM
(150 µl of 12.5 µM + 150 µl of plain media)
6.25µM
(150 µl of 6.25 µM+ 150 µl of plain media)
3.15 µM

Sample preparation

For cytotoxicity studies, 32 mg/ml stocks were prepared using plain media. Serial two folds dilutions were prepared from 320 g/ml to 5 µg/ml using plain media for treatment. (Media used for particular cell lines mentioned in procedure).

32 mg/mL
(20 µl of 32 mg/mL+ 180 µl of Plain media)
3.2 mg/mL
(93.7 µl of 3.2 mg/mL + 206 µl of Plain media)
1mg/mL
(30 µl of 320 µg/mL+ 270 µl of Plain media)
100 µg/mL
(150 µl of 160 µg/mL + 150 µl of Plain media)
50 µg/mL
(150 µL of 80 µg/mL + 150 µL of Plain media)
25 µg/mL
(150 µL 40 µg/mL+ 150 µL of Plain media)
12.5 µg/mL
(150 µL 40 µg/mL+ 150 µL of Plain media)
6.25 µg/ml
(150 µL 40 µg/mL+ 150 µL of Plain media)
3.125 µg/ml
(150 µL 40 µg/mL+ 150 µL of Plain media)

Procedure

To each well of the pre-labelled 96-well microtiter plate, 100 µL of the prepared cell suspension (50,000 cells/well) was added and incubated at 37°C with 5% CO2. After 24h of incubation, the supernatant was removed and the monolayer was rinsed with DMEM. To each pre- designated well, 100µL of test drugs at various concentrations were added and incubated for 24hrs. After incubation, the test solutions in the wells were discarded and 100 µL of MTT reagent (4 mg/10 mL of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37° C in 5% CO2. The supernatant was removed and 100 µL of DMSO was added and the plates were gently shaken to solubilize the formazan crystals. The absorbance was measured using a microplate reader at 590 nm wavelength using a multimode plate reader, Spectra max 13X, Molecular devices. The percentage growth inhibition was calculated using the following formula and the concentration of test drug to inhibit cell growth by 50% (IC50) values is generated from the dose-response curves for each cell line using GraphPad Prism 5.0 software.

Calculating Inhibition:

% Inhibition = ((OD of Control-OD of sample)/OD of Control) x 100.

Statistical evaluation: IC50 Value

The half maximal inhibitory concentration (IC50) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates. How much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor, or microorganism) by half. The IC50 of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonists on reversing agonist activity. IC50 values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. IC50 values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on a sigmoid dose-response curve (variable) and computed using Graph Pad Prism 5 (Graph pad, SanDiego, CA, USA)

Nonlinear regression

In statistics, nonlinear regression is a form of regression analysis in which observational data are modelled by a function which is a nonlinear combination of the model parameters and depends on one or more independent variables. The data are fitted by a method of successive approximation.

Results

The results suggested that the Test Compound showed less inhibition. Hence IC50 cannot be calculated. The reference standard Doxorubicin has shown the IC50 value of 14.33 µM against MCF-7 cells.

Table 2: Cytotoxic effect of test compound on MCF-7 cell lines (n=1)

Concentration	OD	% inhibition
Control	1.144	0.00
5	1.1205	2.05
10	0.9765	14.64
20	0.911	20.37
40	0.8445	26.18
80	0.7166	37.36
160	0.692	39.51
320	0.621	45.72

Table 3: Cytotoxic effect of test compound on MCF-7 cell lines (n=2)

Concentration	OD	%inhibition
Control	1.14	0.00
5	1.1153	2.18
10	0.9721	14.74
20	0.906	20.53
40	0.8338	26.87
80	0.711	37.64
160	0.666	41.58
320	0.618	45.79

Table 4: Cytotoxic effect of test compound on MCF-7 cell lines (n=3)

Conc.	OD	%inhibition
Control	1.14	0.00
5	1.1049	2.93
10	0.9529	16.28
20	0.898	21.10
40	0.8219	27.79
80	0.707	37.88
160	0.658	42.19
320	0.614	46.06

Table 5: Cytotoxic effect of standard doxorubicin on MCF-7 cell lines

	n=1		n=2		n=3			
Conc.	**OD**	**%inhibition**	**OD**	**%inhibition**	**OD**	**%inhibition**	**Average %inhibition**	**IC50**
1.56	0.9969	12.86	0.9934	12.87	0.9894	13.07	12.93	
3.13	0.862	24.65	0.8596	24.60	0.8558	24.81	24.69	
6.25	0.712	37.76	0.7094	37.78	0.7015	38.37	37.97	
12.50	0.6557	42.68	0.6525	42.77	0.6501	42.88	42.78	**14.33**
25.00	0.512	55.24	0.521	54.30	0.515	54.75	54.77	
50.00	0.416	63.64	0.411	63.95	0.412	63.80	63.80	
100.00	0.248	78.32	0.2376	79.16	0.2364	79.23	78.90	

IC50 Plot:

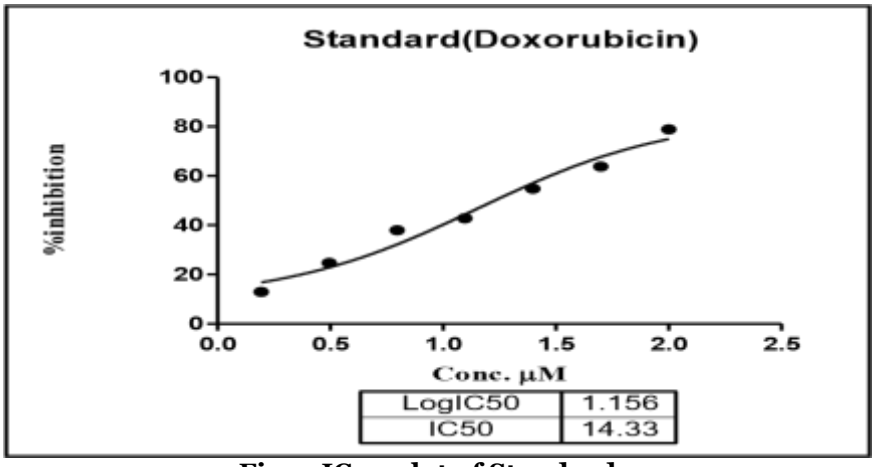

Fig 2: IC50 plot of Standard

Discussion

Clitoria ternatea, commonly known as Shankhpushpi, is a traditional medicinal plant extensively utilized in Ayurvedic medicine for its cognitive and neuroprotective properties. However, recent scientific interest has extended beyond its traditional uses, exploring its potential anticancer properties. Testing *Clitoria ternatea* for cytotoxicity against breast cancer cells is particularly compelling due to several reasons grounded in both ethnopharmacological knowledge and emerging biomedical research. Firstly, the global burden of breast cancer underscores the urgent need for novel therapeutic agents. Despite advances in treatment, breast cancer remains one of the leading causes of cancer-related deaths among women worldwide. Conventional therapies, including chemotherapy, radiation, and surgery, often come with significant side effects and may not be effective against all types of breast cancer. Thus, the discovery of new, effective, and less toxic therapeutic options is paramount. *Clitoria ternatea*, with its long history of medicinal use, represents a promising candidate for such investigations. Secondly, phytochemicals present in *Clitoria ternatea* have shown significant pharmacological activities that could be beneficial in cancer therapy. The plant contains various bioactive compounds such as flavonoids, alkaloids, and glycosides, which have been documented to exhibit antioxidant, anti-inflammatory, and neuroprotective effects. These compounds are known to modulate key cellular pathways that are also implicated in cancer development and progression. For instance, flavonoids have been reported to Induce apoptosis, inhibit cell proliferation, and suppress metastasis in various cancer cell lines, including breast cancer. The rich phytochemical profile of *Clitoria ternatea* thus warrants its evaluation for potential cytotoxic effects on breast cancer cells. Additionally, the safety profile of *Clitoria ternatea* as established through traditional use and preliminary toxicity studies adds to its appeal as a candidate for further investigation. Herbal medicines often offer a more favorable safety profile compared to synthetic drugs, reducing the risk of adverse side effects. This aspect is particularly important in cancer therapy, where the toxicity of conventional treatments can severely impact the quality of life of patients. Furthermore, the exploration of *Clitoria ternatea* for breast cancer treatment aligns with the broader scientific trend of integrating traditional medicine with modern biomedical research. This integrative approach not only validates traditional knowledge but also expands the therapeutic arsenal available for complex diseases like cancer. By conducting rigorous scientific testing, we can uncover the full potential of *Clitoria ternatea*, possibly leading to the development of new, plant-based anticancer drugs that are effective and have minimal side effects.[7,8,9,10]

Clitoria ternatea, known for its extensive medicinal uses, has been documented by Agarwal P et al., for the treatment of hypertension, neurodegenerative diseases, ulcers, high blood pressure, epilepsy, vomiting, diabetes, sunstroke, bleeding, improving memory, and decreasing cholesterol. Given the vast medicinal properties of *Clitoria ternatea*, this study aimed to examine its anticancer activity by testing its cytotoxicity

against breast cancer cells. However, the results revealed that Shankhapushpi is not effective in inducing cytotoxicity in breast cancer cells.[11] Similarly, Debjit Bhowmik et al. documented the use of *Clitoria ternatea* as a traditional Ayurvedic brain tonic for improving memory and treating conditions such as dementia, OCD, phobias, insomnia, anxiety, and stress, as well as Parkinson's and Alzheimer's diseases. Given its vast medicinal properties, this study aimed to investigate its anticancer activity by testing its cytotoxicity against breast cancer cells. However, the results indicated that Shankhapushpi is not effective in inducing cytotoxicity in breast cancer cells.[12] Thakur et al. documented the nootropic, anxiolytic, antidepressant, antistress, neuroprotective, antiamnesic, thyroid-regulating, antioxidant, hypolipidemic, analgesic, antimicrobial, antidiabetic, antiulcer, anticatatonic, reproductive, cardiovascular, anti-addiction, and anthelmintic activities of *Clitoria ternatea*. In line with these comprehensive medicinal properties, our study aimed to investigate its anticancer potential by testing its cytotoxicity against breast cancer cells. However, our results indicated that Shankhapushpi is not effective in inducing cytotoxicity in breast cancer cells.[13] Research by Tamboli, A.M. et al. revealed that *Clitoria ternatea* exhibits cytotoxic and apoptotic effects against the HepG2 cell line. Inspired by their findings, our study examined the cytotoxic effect of *Clitoria ternatea* on MCF-7 breast cancer cell lines. However, our results indicated that Shankhapushpi is not effective in inducing cytotoxicity in MCF-7 cells.[14] Ashpak. M et al. evaluated the anticancer activity of leaf extracts of *Clitoria ternatea* using the Brine Shrimp Lethality Bioassay and found that the ethyl acetate extracts showed significant activity. In contrast, our study revealed that *Clitoria ternatea* is not effective in inducing cytotoxicity in breast cancer cells.[15] Pawar et al., conducted GI50 inhibition study on ethanolic extracts of *Clitoria ternatea* against human breast(MCF-7) and colon cancer (HT-29) cell line. Results revealed that extracts inhibited percent control growth in dose dependent manner for both breast and colon cancer cell line. GI50 (Concentration of drug causing 50% inhibition of cell growth) for breast cancer cell line in *Clitoria ternatea* extract was more than 80 µg/ml. GI50 for colon cancer cell line in all the extracts was found to be more than 80 µg/ml. This result is in contradictory with our result, where root extract of *Clitoria ternatea* was not effective in inducing cytotoxicity in MC-F cell lines.[16]

Conclusion

This study aimed to evaluate the anti-cancerous properties of the root extract of Shankapushpi (*Clitoria ternatea*) on human breast cancer cells (MCF-7). Despite the herb's renowned cognitive-enhancing, anxiolytic, and neuroprotective properties, the root extract did not exhibit significant cytotoxicity or inhibit the proliferation of MCF-7 cells. Various in vitro assays demonstrated that the root extract failed to achieve a half-maximal inhibitory concentration (IC50), suggesting a lack of efficacy under the tested conditions. These findings indicate that Shankapushpi root extract is ineffective in inhibiting breast cancer cell growth, underscoring the need for further research to explore other parts of the plant or different extraction methods that might reveal more promising anti-cancer properties.

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