International Journal of Drug Discovery
ISSN: 0975–4423 & E-ISSN: 0975–914X, Vol. 3, Issue 1, 2011, pp-74-77
Available online at http://www.bioinfo.in/contents.php?id=24

AN INVESTIGATION ON CYTOTOXIC AND ANTIOXIDANT PROPERTIES OF *CLITORIA TERNATEA* L.

VIDHYA RAMASWAMY*, NEETHU VARGHESE, ANCY SIMON
International Centre for Intellectual Training and Empowerment (INCITE), Sri Rams, N.H.Road, Kazhakuttom, Trivandrum, Kerala, India, PIN 695582
*Corresponding author. Email: vidhya.ramaswamy@gmail.com

Received: April 09, 2011; Accepted: June 30, 2011

Abstract- The ethanolic extract of *Clitoria ternatea* L. was evaluated for its *in vitro* cytotoxic and antioxidant activities. The extract showed potent cytotoxic activity in trypan blue dye exclusion method using DLA cell lines with EC50 value of 305µg/ml and exhibited a dose dependent decrease in cell count for all the concentrations tested. The antioxidant activity was evaluated by DPPH free radical method. The extract exhibited potent antioxidant activity with an EC50 of 36.5µg/ml.
Key words: *Clitoria ternatea* L.**,** DLA cell lines, cytotoxic activity, Antioxidant activity, DPPH.

Introduction

Cancer is a disease in which there is uncontrolled multiplication and spread within the body of abnormal forms of body's own cells [1]. Combating cancers is of paramount importance today. An alternative solution to western medicine embodied with severe side effects is the use of medicinal plant preparations to arrest the insidious nature of the disease. Of the 92 anticancer drugs commercially available prior to 1983 in the United States, approved worldwide between 1983 and 1994, approximately 62% can be related to natural origin [2]. Free radical damage may lead to cancer. Antioxidants interact with radicals and may prevent some of the damage by free radicals. Laboratory evidence from chemical, cell culture and animal studies indicate that antioxidants may slow or possibly prevent the development of cancer [3].

Clitoria ternatea L. is a member of the family Papilionaceae; commonly known as 'Aparajita' or Girikarnika'. It is a perennial climber widely used in the traditional Ayurvedic system of Indian medicine for treating a wide variety of ailments. It has slender downy stems with leaves having 5-7 leaflets elliptical to oblong in shape and flowers are usually solitary, bright blue or sometimes white with an orange center and are a very good source of anthocyanins. In the traditional system of medicine, 'Aparajita' is considered as a 'Medhya' drug to improve intelligence and enhance memory function. It is also used in the treatment of chronic bronchitis, dropsy, goiter, leprosy, mucous disorders, sight weakness, skin diseases, sore throat and tumors. The plant contains several glycosides e.g., malvidin-3-β-glycoside, delphinidin-3-β-glycoside.

Clitoria ternatea L. is a very bioactive plant and used in various diseases as folklore medicine [4, 5]. Recent study showed that it has anxiolytic, antidepressant, anticonvulsant and antistress activity [6]. Another study showed that root of *Clitoria ternatea* L. has anti-inflammatory, analgesic and antipyretic properties [7]. Many bioactive compounds have been isolated from different parts of *Clitoria ternatea* L. Recent study showed that malonylated flavonol glycosides were isolated from the petals of *Clitoria ternatea* L.[8]. Another study demonstrated that new anthocyanins, ternatins C1-C5 and 03 and preternatins A3 and C4 were isolated from the young *Clitoria ternatea* L. flowers [9]. It was also reported that new anthocyanins, ternatins A3, B3, B2 and 02 were isolated from *Clitoria ternatea* L. [10]. The deficiencies of the presently available anticancer drugs together with the scientific interest and economical consideration have drawn our attention to the time immemorial, symbolizing the use of plant materials have been used by mankind for treatment of various diseases. In the last two centuries, therapeutic agents have been extracted out of plant sources and many drugs have been found there way into doctor's prescription all over the world [11]. Scientific development in the research field of indigenous medicinal plants is a significant aspect to develop safer anti-cancer principles through their identification, isolation, characterization and biological testing. In the field of cancer chemotherapy, many drugs are available in the world [12]. But due to their potential side effects, clinical toxicities, adverse effect and rapid development of resistance, there is a need to discover novel, safe and most effective drugs for cancer and related diseases. [13,14, 15,16, 17].This present research work investigates the role of *Clitoria ternatea* to be used as effective cytotoxic and antioxidant agents, for treating dreadful diseases spending up new avenues in diseases management for humans.

MATERIALS AND METHODS

Plant material

The leaves of *Clitoria ternatea* have been collected from INCITE garden Trivandrum, Kerala, India during the

month of November 2010 and were dried under shade. The plant was identified by Dr.S.Sreelekha, Taxonomists, Trivandrum and specimen deposited for further investigations.

Preparation of extract

The coarsely powdered shade dried leaves of *Clitoria ternatea* was charged in an aspirator bottle and extracted with ethanol by cold maceration method for 3 days. After decantation and filtering, nearly 80% of the solvent was removed by distillation, over boiling water bath and the remaining under reduced pressure. The extracts so obtained, were further dried in vacuum desicator and used for further studies.

DPPH free radical photometric assay

DPPH (Diphenyl picryl hydrazine) is a free radical at room temperature which produces violet colour in ethanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncoloured solution. The use of DPPH provides an easy and rapid way to evaluate antioxidants. Sample stock solutions (10mg/ml) were diluted to final concentration of 250,125,50,25,10 and 5µg/ml in ethanol. One ml of 0.6mm DPPH ethanol solution was added to 2.5ml of sample solution of different concentrations and allowed to react at room temperature. After 30 minutes, the absorbance values were measured at 518 nm and converted into the percentage antioxidant activity (AA) using the following formula.

AA%=100-{[Absorbance of sample -absorbance of blank]×100/Absorbance of control}.

DPPH solution (1.0 ml, 0.3 mM) and ethanol (2.5 ml) was used as the negative control. The EC50 value was calculated graphically.

Trypan blue dye exclusion method

10mg of the extract was taken in an eppeandorf vial of capacity 1ml and diluted to 6 different concentrations with its duplicate and its control (50%) using alcohol as a solvent and mixed with the help of vortexing machine. Aspirated tumor cells from the peritoneal cavity of mice was obtained from Amala Cancer Research, Thrissur. The procedure was approved by the institutional animal ethics committee. The tumour cells were added to the test tube containing phosphate buffer solution (PBS) which was then dipped in ice. Washed the cells with PBS and centrifuged 3 times. Cells were then suspended in 1 ml 'PBS' and adjusted the cell number to 10 million i.e. 10x106 cells/ml. Checked the cell viability using 'trypan blue' stain (1%) and counted the cells in counting chamber. The experiment was set by incubating the suspension in different concentrations of samples at 37°C for 3 hours. After incubation, added 0.1 ml trypan blue and determined the number of dead cell using haemocytometer.

MTT Assay

Procedure

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

The monolayer cell culture was trypsinized and the cell count was adjusted to 3-lakh cells/ml using medium containing 10% newborn calf serum. To each well of 96 well microtitre plates, 0.1ml of diluted cell suspension was added. After 24 hours, when the monolayer formed the supernatant was flicked off and 100 µl of different test compounds were added to the cells in microtitre plates and kept for incubation at 37ºC in 5 % CO2 incubator for 72 hour and cells were periodically checked for granularity, shrinkage and swelling. After 72 hour, the sample solution in wells was flicked off and 50µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37°C in 5% CO2 incubator. The supernatant was removed, 50 µl of Propanol was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 490 nm. The percentage growth inhibition was calculated using the formula below:

The percentage growth inhibition was calculated using following formula,

% cell inhibition= 100-{(At-Ab)/(Ac-Ab)}x100

Where, At= Absorbance value of test compound, Ab= Absorbance value of blank, Ac=Absorbance value of control

Data interpretation

Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely, a higher absorbance rate indicates an increase in cell proliferation. Rarely, an increase in proliferation may be offset by cell death; evidence of cell death may be inferred from morphological changes.

%cell survival= {(At-Ab)/ (Ac-Ab)} x100

Where, At= Absorbance value of test compound, Ab= Absorbance value of blank, Ac=Absorbance value of control % cell inhibition= 100-cell survival

RESULTS AND DISCUSSION

In the DPPH photometric assay method, the ethanolic extract of *Clitoria ternatea* exhibited a comparable antioxidant activity with that of standard ascorbic acid at varying concentration tested (5, 10, 25, 50,125,250 µg /ml). There was a dose dependant increase in the percentage antioxidant activity for all concentrations tested (Table 1).The extract at a concentration of 5µg/ml showed a percentage inhibition of 18.96±2.02 and for 250 µg/ml it was 89.0±1.64. Ascorbic acid was used as the standard drug for the determination of the antioxidant activity by DPPH method. The concentration of ascorbic acid varied from 1 to 250 µg/ml. Ascorbic acid at a concentration of 1µg/ml exhibited a percentage inhibition of 36.2282±3.514 and for 250 µg/ml 99.124±0.282 (Table 2). A graded increase in percentage of inhibition

was observed for the increase in the concentration of ascorbic acid. The EC50 value of ascorbic acid was found to be 6.1 µg/ml. All determinations were done in duplicate and the mean values were determined. An increased EC50 value was observed (36.5µg/ml) for the plant extract when compared with standard drug ascorbic acid (6.1µg/ml) (Table 1 & 2) Estimation of the cytotoxic activity was done by trypan blue dye exclusion method using DLA cell lines. The various concentrations of plant extracts used were 10, 50, 100, 200, 500 µg/ml and control (without extract). A decrease in the cell count was observed with the increase in the concentration of the extract. There was a dose depended increase in the cytotoxic activity for all the concentrations tested. The extract at a concentration 10 µg/ml showed 1% reduction in cell count, and 95% reduction was observed for 500 µg/ml concentrations. The IC50 value was found to be 305 µg/ml. The cytotoxicity activity was analyzed using MTT assay and the concentration of Hela cells at decreasing gradient of 10,5,2.5,0.625,0.312,0.156,0.078,0.0391and 0.0196 were tested. The absorbance and percentage of inhibition ranged between 1.519to2.005and 62.61 to 38.28 respectively. The maximum percentage of cytotoxic inhibition was reported for 10 µg/ml concentration and the minimum percentage of inhibition was for 0.0196 µg/ml concentration. The IC50 value for *Clitoria ternatea* was 265.0 when observed in MTT assay analysis. The cytotoxic activity decreases gradually from10 µg/ml concentration to 0.019610 µg/ml concentration from 62.61% to 38.28 % respectively (Table 3 & 4).

Plants have a great potential for producing new drugs for human benefit. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and even infectious diseases. According to a report of World Health Organization, more than 80% of world's populations depend on traditional medicine for their primary health care needs. The demand for more and more drugs from plant sources is continuously increasing. It is therefore essential for systematic evaluation of plants used in traditional medicine for various ailments. The increased interest in plant derived drugs is mainly because of the wide spread belief that 'herbal medicine' is safer than costly synthetic drugs which possesses side effects. Hence, there is need to screen medicinal plants for promising biological activity. Further, there is a continuous development of resistant strains which pose the need for search and development of new drug to cure diseases.

Plant substrates continue to serve as viable source of drugs for the world population and several plant based drugs are in extensive clinical use. Agents capable of inhibiting cell proliferation, inducing apoptosis or modulating signal transduction are currently used for the treatment of cancer. The use of multiple chemo preventive agents or agents with multiple targets on cancer cells are considered to be more effective in cancer treatment.

Recent reports have cited that plants and its components could act as tumor suppressor, apoptotic inducer in cancer cells and the most commonly used herbal medicine have tumor suppressing activity, interfere with cell cycle progression, enhance immune activity and suppress tumor angiogenesis(15). Likewise the aqueous extract of *Helixanthera parasitica* is also reported to have great cytotoxic potential (16). In the present study, the *Clitoria ternatea extracts* is well correlated with previous reports from different plant extracts on cancer suppressing activity or anti carcinogenic activity.

CONCLUSION

On the basis of the above results it can be concluded that the ethanolic extract possess significant anticancer and antioxidant activities studied by *in vitro* models. The presence of flavonoids and related phytoconstituents may be responsible for the activity. Further investigations are required to find active component of the extract and to confirm the mechanism of action. Further studies warranted for isolation of the constituents responsible for the activity and also to explore the exact mechanism of action of the activity in *Clitoria ternatea*

REFERENCES

[1] Rang H.P., Dale M.M., Ritter J.M., Moore P.K. (2003) *Pharmacology 5th ed. Churchill Livingstone: Elsvier*, p.693.

[2] Gordon M.C., Newman D.J. (2001) *Pharm. Biol*, 39:8–17.

[3] Blot W.J., Lyji., Taylor P.R. (1993) *Natl cancer inst*, 85(3), 1483-1491.

[4] Mclanghlin J.L. (1990) *Bench-top bioassay for the discovery of bioactive compounds in higher plants*.

[5] Evans W.C. (2002) *Pharmacognosy; W. B. Saunders; 15th edition, Edinburgh*, 475.

[6] Jain N.N. (2003) *Pharmacol Biochem Behav*, 75(3): 529-36.

[7] Devi B.P. (2003) *FitoterapiaJum*, 74(4): 345-9.

[8] Kazuma K. (2003) *Phytochemistry*, 62(2):229-37.

[9] [Terahara N. (1998) *J Nat Prod*, 61(11): 1361-7.

[10] Terahara N. (1998) *J Nat Prod* 61(11): 1361-7.

[11] Hardman J.G., Limbard L.E., Goodman & Gilman's (1996) *The Pharmacological Basis of therapeutics, McGraw-Hili, London, 9th edition*, :1228-29.

[12] Hardman J. G., Limbard L. E., Goodman & Gilman's. (1996) *The Pharmacological Basis of therapeutics, McGraw-Hili, London, 9th edition* 1227-29.

[13] Hardman J.G., Limbard L.E., Goodman & Gilman's. (1996) *The Pharmacological Basis of therapeutics, McGraw-Hili, London, 9th edition*: 1238-75.

[14] Harrap K.R., Davis W., Calvert A.H. (1984) *Cancer chemotherapy and selective drug*

development, Martinus Nijhoff Publishing, USA: 5-10.
[15] Devita V.T. (1983) *Cancer* 51: 1209-1220.
[16] Hardman J.G., Limbard L.E., Goodman & Gilman's. (1996) *The Pharmacological Basis of therapeutics, McGraw-Hili, London, 9th edition*, 1237-58.
[17] Evans W.C. (2002) *Pharmacognosy; W. B. Saunders; 15th edition; Edinburgh* 137-40.

Table 1- Antioxidant activity of Clitoria ternatea extract by DPPH free radical method

Concentration µg/ml	% Ascorbic acid	EC50
5	18.96 ± 2.02	36.5µg / ml
10	26.62 ± 0.92	
25	7.12 ± 1.11	
50	88.31 ± 1.23	
125	88.58 ± 0.08	
250	89.0 ± 1.64	

Table 2- Antioxidant activity of Ascorbic Acid by DPPH free radical method

Concentration µg/ml	% Ascorbic acid	EC50
5	36.23 ± 1.142	6.1 µg / ml
10	41.63 ± 0.284	
25	84.24 ± 0.321	
50	84.13 ± 0.114	
125	95.14 ± 0.074	
250	99.12 ± 0.282	

Table 3- Cytotoxic activity using DLA cells lines by Trypan Blue Dye Exclusion Method

Concentration µg/ml	DLA % Cytotoxic activity	IC50
10	1 ±0.525	305 µg / ml
50	2 ±0.623	
100	12 ±0.110	
200	25 ±0.089	
500	95±0.244	
Control	0	

Table 4- Determination of cytotoxicity by MTT assay

Concentration µg/ml	Hela		IC50
	Absorbance	% Inhibition	
10	1.519	62.61	
5	1.560	60.56	
2.5	1.62	57.54	
1.25	1.63	57.04	
0.625	1.658	55.62	265.0
0.312	1.735	51.79	
0.156	1.745	51.29	
0.078	1.918	42.66	
0.0391	1.93	42.03	
0.0196	2.005	38.28	