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**Research Article** 

# ANTI-CANCER ACTIVITY OF Datura metel ON MCF-7 CELL LINE

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## ABSTRACT

Objective: Current clinical trends involve the usage of plants as therapeutic agents in a wide range of applications. Present investigation is focused on the anticancer activity of the methanolic extract of *Datura metel* against MCF-7 cell line.

Materials & Methods: The study was facilitated by collecting the plant sample and subjected to methanol extraction using Soxhlet apparatus. The anticancer activity of the extracted sample against MCF-7 cell line was examined by MTT assay.

Results: The study confirms that the leaf extract of *Datura metel* has pronounced anticancer potential against MCF-7 cell lines while compared to that of the stem extract.

Conclusion: The plant investigated possesses remarkable anticancer activity and hence isolation of the compound contributing to the activity may lead to develop at a novel and natural phytomedicine for the disease.

Keywords: Datura Metel, MCF-7 cell line, MTT assay, anti-cancer activity

## INTRODUCTION

A report by World Health Organization states that around 80% of people in the world rely on phytomedicine [11] and 33% of drugs used are from plant sources [2]. According to Hartwell, around 3000 species of plants are currently being used in cancer therapy [3]. One such plant with anticancer potential is *Datura metel* which is from *solanaceae* family [4-6]. The species has common names such as thorn apple and downy datura. In Tamil, it is commonly called as Oomatthai, Karuvoomatthai [7]. The plant species is rich in various kinds of alkaloids, such as hyoscine, fastusine, hyoscyamine, littorine, valtropine and acetoxytropine. It also has many withonilides with anticancer properties [8,9] and calystigines with glycosidase inhibitory property [10].

The prevalence of breast cancer in Indian women is more at the age of forty [11]. The incidence of breast cancer has been increasing worldwide for many decades [12] with Asian countries attaining highest incidence rate [13]. Some breast tumors stay resistant to conventional treatment [14,15] and may have many side effects which affect the quality of the treatment [16]. Skirmishing with such a dreadful disease as a treatment must be considered with high importance. Surgical treatments are in need by the specialized proficient surgeons in the area of surgical oncology [17,18]. Existing radiation oncology infrastructure is not sufficient for the most developing countries [19]. According to the data of world Health organization, chemotherapy is needed for more than 90% of people affected with breast cancer.

Nowadays, researchers focus greatly on folk medicine to develop better drugs for cancer.<sup>1</sup> The traditional users own only ideas on the identification of the plant and dosages through personal practices but are not with an awareness on scientific reasons behind its medicinal uses [1,20]. Owing to the growth in medical perception, plant derived compounds can be designed as drugs for diseases. The present investigation focused on the determination of the anticancer effect of the plant species *Datura metel* on breast cancer MCF-7 cell lines , which was compared to the effects on control Vero cell lines.

## **METERIALS AND METHODS**

Collection of plant samples

Healthy fresh plants of *Datura metel* species were collected from Vallam village at a close proximity to Thanjavur district during the month of January in the year 2012. The plant species were recognized and authenticated by a taxonomist. Different parts of the

plants were isolated and air dried at room temperature. The dried samples were ground into powder and the powdered samples were subjected to Soxhlet extraction with methanol as solvent. The extracts were collected and stored in air tight containers for further studies.

#### Phytochemical analysis

The plant samples were subjected to phytochemical analysis using the standard protocol [21, 22].

## MTT Assay

After the extraction of the sample, the viability of the cells were determined through MTT assay[23,24]. MCF-7 cells and VERO cells were collected and washed twice with PBS and 10  $\mu$ l of MTT reagent (5 mg/mL in PBS) were added in the wells. The plates were kept for incubation for 4 h. The incubated cells were washed twice with PBS and DMSO (100 $\mu$ L/well) reagent which dissolved the insoluble crystalline formazan product. The efficacy of the sample was determined based on the reduced dye at 570 nm by UV spectrophotometer [25]. The effect of the samples on the proliferation of MCF-7 cell lines and VERO cell lines were expressed as the % cell viability, using the following formula:

% cell viability = A570 of treated cells / A570 of control cells  $\times$  100%[26].

#### RESULTS

The leaf extract possessed more anticancer potential and composed of phytocomponents like alkaloids, sterols saponins, phenols, tannins and flavonoids. (table.1)

## Table1: The detected compound in the plant sample.

S. No.	Test	Leaves	Stem
1	Alkaloids	+	+

2	Terpenoid and steroid	+	+
3	Flavonoid	+	+
4	Phenolic	+	-
	compounds		
5	Saponins	+	-
6	Tannins	+	-
7	Glycosides	+	-

Effects of leaf and stem extracts on the cell lines used have been portrayed in figures 1-4.



## Fig1: Anticancer effect of Datura metel leaf extract on Vero cell line

Figure 1(a) Normal VERO cell line,(b) Toxicity at 1000 µg/ml, (c)Toxicity at 125 µg/ml (d)Toxicity at 62.5 µg/ml, (e)Toxicity at 31.2 µg/ml.

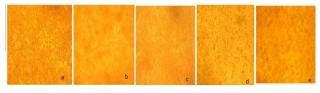


Fig.2: Anticancer effect of Datura metel leaf extract on MCF-7 cell line

Fig.2 (a)Normal MCF-7 cell line, (b) Toxicity at 1000  $\mu$ g/ml, (c)Toxicity at 125 µg/ml, (d)Toxicity at 62.5 µg/ml (e) Toxicity at 31.2 µg/ml

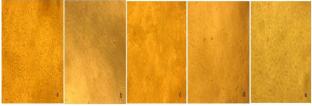


Fig.3: Anticancer effect of Datura metel stem extract on Vero cell line

Fig.3(a) Normal VERO Cell line,(b) Toxicity at 1000 µg/ml, (c)Toxicity at 250 µg/ml (d) Toxicity at 62.5 µg/ml, (e)Toxicity at 31.2 µg/ml.



Fig.4: Anticancer effect of Datura metel stem extract on MCF-7 cell line

Fig.4(a) Normal MCF-7 cell line 2, (b) Toxicity at 1000 µg/ml ,(c)Toxicity at 250 μg/ml, (d) Toxicity at 62.5 μg/ml, (e)Toxicity at 31.2 μg/ml.

Concentrations of the samples required to inhibit 50% (IC<sub>50</sub>) of the viability of the cells were determined and highlighted in tables 2-5.

Table2: Anticancer effect of the stem extract on Vero cell line.

S.No	CONCENTRATION	ABSORBANCE	CELL VIABILITY
	(µg/ml)	(0.D) nm	(%)
1	1000	0.07	15.2
2	500	0.12	26.0
3	250	0.18	39.1
4	125	0.22	47.8
5	62.5	0.27	58.6
6	31.2	0.33	71.7
7	15.6	0.39	84.7
8	7.8	0.42	91.3
9	Cell control	0.46	100
Table3: Anticancer effect of leaf extract on Vero cell line.			
S.No	CONCENTRATION	ABSORBANCE	CELL VIABILITY
	(µg/ml)	(0.D) nm	(%)
1	1000	0.06	13.0
2	500	0.10	21.7
3	250	0.19	41.3
4	125	0.23	50.0
5	62.5	0.29	63.0

0.46 Table 4: Anticancer effect of the stem extract on MCF-7 cell line.

0.34

0.40

0.43

73.0

86.9

93.4

100

S.No	CONCENTRATION (µg/ml)	ABSORBANCE (O.D) nm	CELL VIABILITY (%)
1	1000	0.02	4.1
2	500	0.10	20.8
3	250	0.16	33.3
4	125	0.22	45.8
5	62.5	0.27	56.2
6	31.2	0.31	64.5
7	15.6	0.36	75.0
8	7.8	0.40	83.3
9	Cell control	0.48	100

Table5: Anticancer effect of the leaf extract against MCF-7 cell
line

S.No	CONCENTRATION (µg/ml)	ABSORBANCE (O.D) nm	CELL VIABILITY (%)
1	1000	0.08	8.3
2	500	0.13	16.6
3	250	0.19	27.0
4	125	0.23	39.5
5	62.5	0.23	47.9
6	31.2	0.29	60.4
7	15.6	0.33	68.7
8	7.8	0.38	79.1
9	Cell control	0.48	100

## DISCUSSION

31.2

15.6

7.8

Cell control

6

7

8

9

The phytochemical analysis of the methanolic extract of the plant reveals that the plant has components such as alkaloids, terpenoid, steroids, flavonoid, phenolic compounds, saponins, tannins and glycosides. It was observed that the ethanol extract of the leaves had high anticancer activity than the stem extract on both Vero and MCF-7 cell lines as they had low  $IC_{50}$  values compared to the latter (tables 2 -5). The withonilides which are steroidal lactones present in the plant have a have been reported to have a high anticancer activity against colorecto carcinoma (HCT-116) cell line [31, 32]. Many solanaceae species are rich in calystegines, which have glycosidase inhibiting properties against cancer [6]. The results obtained hence confirm that the plant has signifcant anticancer potential as stated in the other reports about the species [26-30]. Manv other phytocomponents extracted can also be expected to contribute to the anticancer activity. Cysteine methylation is associated with many diseases including breast cancers [33]. In future, we also plan to study the mechanism of the anticancerous potential of the plant in

particular we will investigate whether the plant extract influences DNA methylation and gene expression in breast cancer.

## CONCLUSION

The methanolic leaf extract of the *Datura metal* is considered to have a high anticancer potential compared to stem. The outcome of the present study encourages carrying out further investigation by isolating a particular component with anticancer activity so as to design a specific drug for the disease. In future, we also plan to study the mechanism of the anticancerous potential of the plant, in particular, we will investigate whether the plant extract influences DNA methylation and gene expression in breast cancer.

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#### REFERENCES

- Gurib-Fakim. Medicinal plants: Traditions of yesterday and drugs of tomorrow, Molecular Aspects of Medicine, 2006; 27:1–93.
- Srinivasan D, Nathan S, Suresh T, Lakshmana P. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J Ethnopharmacol. 2001; 217-220.
- 3. Hartwell JL. Plants used against cancer a survey. Lawrence, MA. Quarterman Publications, 1982; pp 438-39.
- 4. Duke J, Handbook of medicinal herbs. **CRC Press Inc**. Boca Raton.1985.
- Hussein K. Medicinal plants in Libya. Arab Encyclopedia House, Tripoli. 1985.
- 6. Jacob GS. Glycosylation inhibitors in biology and medicine. **Current biology** 1995; 5:605-11.
- 7. Khare CP, (Ed.) Indian Medicinal Plants. An Illustrated Dictionary With Pictures of Crude Herbs 2007; 123.
- Afshypuor S, Mostajeran A, Mokhtary R. Variations of scopolamine and atropine in different parts of Datura metel during development. Plant Med. 1995; 61:383-4.
- 9. Monira KM, Munan SM. Review on Datura metel a potential medicinal plant. **Globel J Res Med Plant Indegen Med** 2012; 1:123-32.
- Ghani A. Medicinal plants of Bangaladesh with chemical constituents and uses 2<sup>nd</sup> ed. Dhaka: Asiatic society of Bangadesh: 2003
- Murthy NS, Agarwal UK, Chaudhry K, Saxena S. A study on time trends in incidence of breast cancer –Indian scenario. Eur J Cancer Care 2007; 16:185-6.
- Hortobagyi GN, de la Garza Salazar J, Pritchard K, Amadori D, Haidinger R, Hudis CA, Khaled H, Liu MC, Martin M, Namer M, O'Shaughnessy JA, Shen ZZ, Albain KS. The global breast cancer burden: variations in epidemiology and survival. Clin Breast Cancer 2005; 6: 391-401.
- Green. M, Raina. V. Epidemiology screening and diagnosis of breast cancer in the Asia–Pacific region: current perspectives and important considerations. Asia Pac J Clin Oncol 2011; 4: 5-13.
- Higgins MJ, Baselga J. Targeted therapies for breast cancer. J Clin Invest 2011;121:3797–803.
- 15. Premalatha Balachandran , Rajgopal Govindarajan, Cancer an ayurvedic perspective, **Pharmacological Research** 2005;19–30.
- 16. Deo SVS. Challenges in the treatment of breast cancer in developing countries. **Nat Med J India** 2010; 3: 129-31.
- Ravichandran R. Has the time come for doing away with Cobalt-60 teletherapy for cancer treatments? J Med Phys,Asian Pacific Journal of Cancer Prevention 2012; Vol 13: 63-5.

- Khokar A. Breast Cancer in India: Where Do We Stand and Where Do We Go? Asian Pacific Journal of Cancer Prevention 2012;13(10):4861-4866.
- Kilani AM . Antibacterial assessment of whole stem bark of Vitex doniana against some Enterobactriaceae. African Journal of Biotechnology 2006; 5 (10): 958-959.
- Kokate, CK., Purohit AP Gokhale SB. Practical Pharmacoghnocy, Ed.30, Nirali Prakashan, Pune, India 2004; pp: 593-597.
- Sofowara A. Medicinal Plants and Traditional Medicine in Africa. 3 Ed. Spectrum Books Limited, Ebadan, Nigeria 2008; pp:199-204.
- 22. Borenfreund E, Babich H, Martin-Alguacil N. Comparison of two in vitro cytotoxicity assays: the neutral red (NR) and tetrazolium (MTT) tests. **Toxicol Vitro** 1988; 2: 1-6.
- 23. Mossman. T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
- 24. Edmondson J. et al. A rapid and simple MTT-based spectrophotometric assay for determining drug sensitivity in monolayer cultures. J Tissue Cult. Methods 1988 11:15-17.
- 25. Dhiman A, Lal R. Phytochemical and Pharmacological status of *Datura fastuosa Linn*. **International Journal of Research in Ayurveda and Pharmacy** 2011; 2(1): 145-150.
- Donatus EO, Ephraim C. Isolation, characterization and antibacterial activity of alkaloid from Datura metel Linn leaves. African Journal of Pharmacy and Pharmacology 2009; 3(5): 277-281.
- 27. Akharaiyi. FC. Antibacterial, Phytochemical and Antioxidant activities of Datura metel. **International Journal of Pharm Tech Research** 2011; 3(1): 478-483.
- John De Britto A, Herin Sheeba Gelin D. Datura metel Linn, A plant with potential as antibacterial agent. International Journal of Applied Biology and Pharmaceutical Technology 2011 a; 2(2): 429-433.
- Arshad J, Sobiya S, Shazia S. Herbicidal Activity of *Datura Metel L.* against Phalaris minor Retz. Pak. J.Weed Sci. Res 2008; 14(3-4). 209-220.
- Ma L, Xie CM, Li J, Lou FC and Hu LH Daturametelins H, I, and J:Three New Withanolide Glycosides from *Datura metel L*. Chem. & Biodiv 2006; 3:180–186.
- 31. Glotter E, Nat. Prod. Rep1991; 8: 415.
- Nash RJ, Rothschild M, Porter EA, Watson. A, Waigh. RD and Waterman PG. Calystegines in *Solanum* and *Datura* species and the death's-head hawk-moth (*Acherontia atropus*). Phytochem 1993; 34: 1281–1283.
- Basheer NB, Rajasree S, Laxmi A, Harshiny M, Kaliaperumal R, Shanmugam K, Conventional and nano techniques for DNA methylation profiling. J. Mol. Diag. 2013;15: 17-26.