# In vitro Cytotoxicity studies on *Ipomoea digit at a* -An important Traditional Medicinal Plant

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**Abstract**

Cancer is a second leading cause of death globally. *Ipomoea digitata* also known as milk yam is an extensive perennial climber, large glabrous liana belonging to Convolvulaceae family. Pharmacological studies revealed that plant possessed antioxidant, antibacterial, antidiabetic, antihypertensive and antihyperlipidaemic properties. However, to the best of our knowledge till date, there are no available studies on the cytotoxicity of this plant. Thus, this study was aimed to evaluate in vitro anticancer activity of *Ipomoea digitata* against A549, MCF-7 and Hep G2 human cancer cell lines using MTT assay. The Phytochemical investigation confirmed the presence of carbohydrates,alkaloids, tannins, flavonoids, phytosterols, saponins, The ethanol extract significantly reduced the percent viability of A549, MCF-7 and Hep G2 cancer cell lines with IC50 value 69.94±1.08µg/ml for A549, 81.94±1.01µg/ml for MCF-7 and 47.28±0.65µg/ml for Hep G2.

**Keywords:** In vitro anticancer activity, *Ipomoea digitata*, MTT assay
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## Introduction

Cancer is a recognized as a non-communicable disease which lacks universal solution and is second leading cause of death globally[1].It is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body[2]. Cancer development is a multistage process resulting from the transformation of normal cells into tumor cells including transition from a pre-cancerous lesion to a malignant tumor state. These changes are the result of the interaction between a person's genetic factors with physical (ultra violet radiation), chemical (tobacco and asbestos) and biological (virus and bacteria) factors. In cancer there may be deregulation of single and/ or multiple cellular mechanisms such as, cell division, apoptosis which are required for normal growth and proliferation of healthy cells[3]. Despite of spending billions of dollars on cancer research and development, we do not understand cancer in detail and disease still impacts millions of patients all over the world[4]. The current treatment for all types of cancer includes chemotherapy, radiation therapy, hormonal therapy and surgery[3,5].The disadvantage of chemo-preventive practices includes side effects, multidrug resistance, suppression of the immune system and destruction of healthy cells and tissues therefore it has become necessary to hunt for an alternative anti-cancer treatment[6].Thus, for the development of more effective and safer drugs to inhibit the onset of cancer new anticancer agents from natural products are expected to play an important

role[7].Medicinal plants are considered as an important source for the discovery and development of new drugs. Plants contain diverse active potentially important compounds that are used for development as therapies for many human diseases including cancers[8]. There has been a breakthrough in the use of medicinal plants in the field of oncology in recent decades. A review states that, 47% of the drugs used in the treatment of cancers are either natural products or their semi-synthetic derivatives[9]. Despite the great importance of natural products in anti-cancer development, it is estimated that only 2% of the natural products were investigated for anti-cancer[10].Several plant-based molecules such asvinblastine, vincristine, camptothecin, epipodophyllotoxin, taxanes and their derivatives are used clinically to treat various types of cancers[11].

The genus Ipomoea have been in continuous use for different purposes, such as, nutritional, medicinal, ritual and agricultural. The phytochemistry of the Ipomoea genus has been studied since 1950. Some species of Ipomoea showed antimicrobial, analgesic, spasmolytic hypotensive, psychotomimetic and anticancer activities[12].*Ipomoea digitata* also known as milk yam is an extensive perennial climber, large glabrous liana belonging to Convolvulaceae family.The plant possess five to seven lobed leaves, bell shaped pink coloured flowers and tuberous root system[13].It is generally referred as Bilai-kand, Bhui-khola, Bhumi-kumra, Bhumi-kushmanda, Ksheervidari, Payasvinee, in various languages[14]. Traditionally its tubers are used as tonic and aphrodisiac. The plant is also used in uterine pain, lactation, gastric ulcer, blood dysentery, infertility, high blood pressure and heart diseases[15].Pharmacological studies revealed that this plant possessed antidiabetic[16,17], antihyperlipidaemic[18], antioxidant[19] and antihypertensive[20]properties. The present study was aimed to evaluate preliminary phytochemical and in vitro anticancer activity of Ipomoea digitata against A549, MCF-7 and Hep G2 human cancer cell lines.

**Collection, authentication of plant and preparation of extract**

The plant was collected from Instructional Farm, College of Agriculture, Vellayani, Thiruvananthapuram, Department of plantation crops and Science college of Agriculture Vellayani, Thiruvananthpuram. The plant was authenticated by Dr. Dongarwar, Department of Botany, R. T. M. Nagpur University Campus, Nagpur. The herbarium sheet having voucher specimen number 10576 was deposited in Department of Botany, R.T.M. Nagpur University, Nagpur for future reference. The tubers were dried in shade, powdered and stored in airtight container for extraction. The air dried tuber powder (1 kg) was defatted with petroleum ether by hot percolation using soxhlet apparatus for 72 hrs. The marc left after the petroleum extract was dried and subsequently extracted with ethanol 95 % at $60^0$ - $70^0$C up to 72 hours in soxhlet apparatus.

**Phytochemical analysis**

The ethanolic extract(EE)was subjected to different qualitative tests to indicate presence or absence of different phytochemical constituents like alkaloids, glycosides, phenolic compounds, flavonoids, saponins, sterols, tannins, fixed oils and fats, protein and amino acids, gum and mucilage and carbohydrates by adopting the standard procedure[21].



**Culturing of cell lines**

The Human breast cancer (MCF 7), human lung adenocarcinoma epithelial cell line (A549), and human liver cancer cell line (Hep G2) were cultured in DMEM with low glucose and supplemented with 10 % FBS and 1% penicillin-streptomycin antibiotic-antimycotic 100X solution and incubated in humidified atmosphere of 5 % $CO_2$ and 37 °C. The culture medium was changed every two days.

**MTT Cell Viability Assay**

**Preparation of working concentrations of plant extract:**

Working concentrations of extracts of *Ipomoea digit at a* was prepared by incorporating desired quantity of the alcoholic extract into di-methyl

sulfoxide prior to the experiment. The reactant mixtures were diluted with media and cells were treated with different concentration ranges (15.625–500µg/ml)

**Procedure**

The cells were seeded in a 96-well flat-bottom micro plate and incubated for 12 hrs and maintained at 37ºC in 95% humidity and 5% $CO_2$. Different concentration (100, 50, 25, 12.5, 6.25, 3.125µg/ml) of ethanolic extract of *Ipomoea digitata* was added to each well and incubated for another 48 hours. The cells in well were washed twice with phosphate buffer solution, and 20 µl of the MTT staining solution (5 mg/ml in phosphate buffer solution) was added to each well and plate was incubated at $37^0$C for 4hrs in dark. This results in formation of formazan crystals. Following this 100 µl of di-methyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals, and absorbance was recorded at 570 nm using micro plate reader[22, 23].

Formula:

Surviving cells (%) = Mean OD of test compound/Mean OD of Negative control x 100

**Results**

The preliminary phytochemical studies on plant revealed presence of carbohydrates, alkaloids, flavonoids, tannins, phytosterols and saponins.

**Table 1: Showing cell viability values at various concentration ethanolic extract of *Ipomoea digitata* for MTT assay comparing with standard drug Doxorubicin against A549 cell lines**

| **Cell viability of A549** | | | | | | |
|---|---|---|---|---|---|---|
| **Concentration (µg/ml)** | **EE of *Ipomea digitata*** | | | **Doxorubicin** | | |
| 100 | 46.59 | 45.79 | 44.6 | 29.37 | 26.19 | 28.17 |
| 50 | 59.37 | 59.76 | 58.97 | 30.95 | 32.14 | 32.54 |
| 25 | 71.35 | 71.35 | 70.56 | 34.13 | 34.92 | 35.32 |
| 12.5 | 83.33 | 82.54 | 83.73 | 38.49 | 38.89 | 37.7 |
| 6.25 | 86.51 | 86.9 | 87.7 | 41.67 | 43.25 | 40.87 |
| 3.125 | 88.1 | 88.49 | 90.08 | 53.17 | 56.75 | 58.33 |
| Negative control | 100 | | | 100 | | |
| $IC_{50}$ value | 69.94 | | | 7.33 | | |
| Standard deviation | 1.08 | | | 0.33 | | |

**Table 2: Showing cell viability values at various concentration ethanolic extract of *Ipomoea digitata* for MTT assay comparing with standard drug Doxorubicin against MCF-7 cell lines**

| **Cell viability of MCF-7** | | | | | | |
|---|---|---|---|---|---|---|
| **Concentration (µg/ml)** | **EE of *Ipomea digitata*** | | | **Doxorubicin** | | |
| 100 | 49.08 | 50 | 49.23 | 17.23 | 17.85 | 17.08 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 50 | 61.08 | 61.54 | 61.23 | 22.31 | 22.15 | 21.85 |
| 25 | 76.15 | 75.23 | 74.62 | 24.77 | 25.85 | 25.23 |
| 12.5 | 84.46 | 83.77 | 82.69 | 28.77 | 28.31 | 28.46 |
| 6.25 | 88.15 | 89.46 | 90.77 | 29.69 | 29.08 | 29.69 |
| 3.125 | 94.46 | 93.54 | 94.15 | 61.69 | 62 | 62.62 |
| Negative control | 100 | | | 100 | | |
| $IC_{50}$ value | 81.94 | | | 4.79 | | |
| Standard deviation | 1.01 | | | 0.03 | | |

**Table 3: Showing cell viability values at various concentration EE of *Ipomoea digitata* for MTT assay comparing with standard drug Doxorubicin against Hep G2 cell lines**

| **Cell viability of Hep G2** | | | | | | |
|---|---|---|---|---|---|---|
| **Concentration (µg/ml)** | **EE of *Ipomea digitata*** | | | **Doxorubicin** | | |
| 100 | 28.62 | 27.46 | 26.31 | 17.23 | 17.85 | 17.08 |
| 50 | 51.54 | 54.38 | 53.08 | 22.31 | 22.15 | 21.85 |
| 25 | 69.85 | 69.62 | 71.23 | 24.77 | 25.85 | 25.23 |
| 12.5 | 74.46 | 75.15 | 74.92 | 28.77 | 28.31 | 28.46 |
| 6.25 | 85.38 | 85.31 | 84.31 | 29.69 | 29.08 | 29.69 |
| 3.125 | 90.85 | 91.54 | 92 | 61.69 | 62 | 62.62 |
| Negative control | 100 | | | 100 | | |
| $IC_{50}$ value | 47.28 | | | 0.65 | | |
| Standard deviation | 4.79 | | | 0.03 | | |

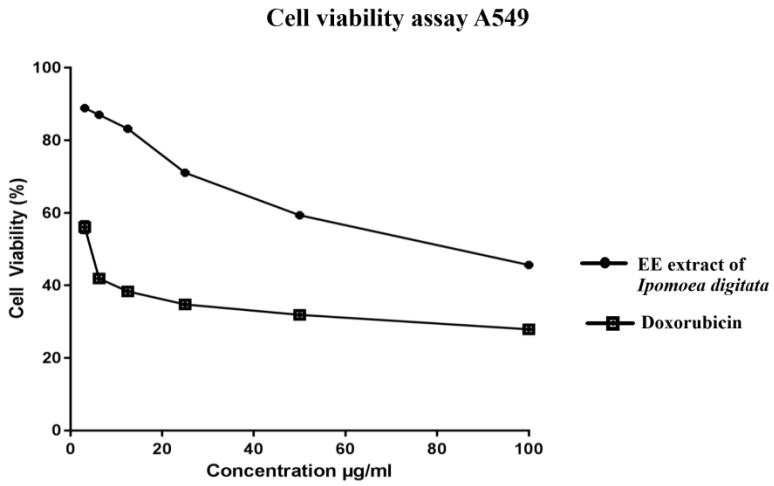


**Figure 1 Percentage of cell growth inhibition of various concentration of *Ipomoea digitata* against A549 cell lines**

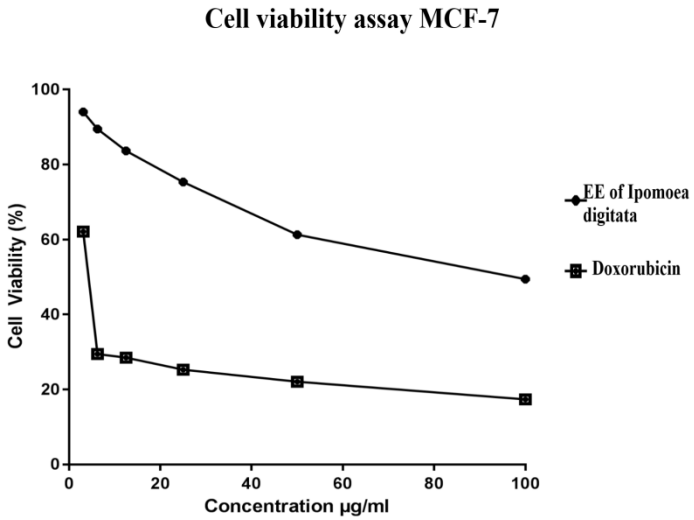




**Figure 2 Percentage of cell growth inhibition of various concentration of *Ipomoea digitata* against MCF-7cell lines**

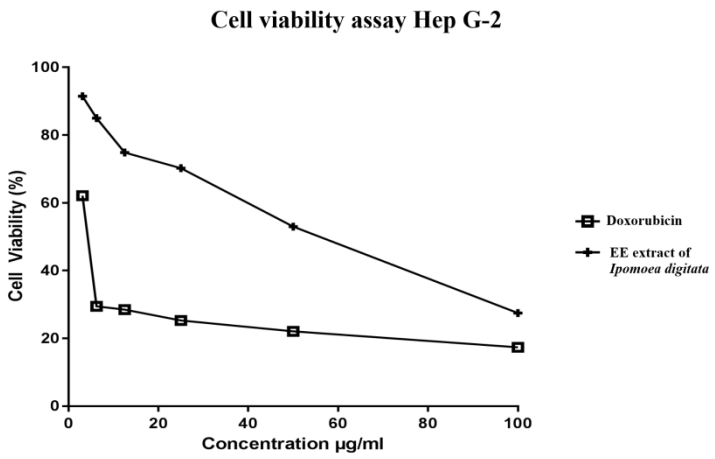


**Figure 3 Percentage of cell growth inhibition of various concentration of *Ipomoea digitata* against Hep G-2cell lines**

**Discussion**

Plants and plant products are used as medicine worldwide, from ages. Various studies have confirmed that plants play a crucial role in anti-cancer research. Effective anti-cancer drugs can be derived from plant sources with an elucidated mechanism of action, by employing different modern technologies. Many researchers have spent tremendous amount of time and resources in studying the importance of medicinal plants in recent years. The pharmacological importance of medicinal plants can never be underestimated accounting to their easy to handle, high rate of efficacy, affordable by price, clear abundance in nature and most important near negligible side effects. Due to the fact that these plants have been used for centuries as remedies against different ailments in the traditional medicinal system, research on them makes it more trustworthy, as fine tuning the components of each medicinal plant would significantly increase their efficacy. The present work involves preliminary in vitro anticancer activity of *Ipomoea digit at a* wherein the plant was evaluated first time for anticancer activity against different cell lines. Ethanolic extract of the plant demonstrated significant anticancer effect on all the cell lines under study but the extract was found to be more effective against human liver cancer cell line. This effect can be attributed to the presence of phytochemical compounds present in the plants.



**Conclusion**

In an attempt to search for safer, more effective and lower cost anticancer treatment the recent research area is targeting cancer with phytochemicals. The present study aimed to determine anticancer effect of *Ipomoea digitata***.** The results revealed that ethanolic extract of the plant contains cytostatic chemicals, which possess selective anti-cancer activity against cancer cell lines, particularly human liver cancer cell line. In order to characterize potential antitumor components of the plant further studies are to be conducted along with confirmatory in vivo studies.

**Refrences**

1. Kumar S., R. Singh, D. Dutta, S. Chandel, A. Bhattacharya, V. Ravichandiran & S. Sukla (2022) *Molecules*, 27: 8222: 1-21

2. Alasmary F.A.S., A.S. Awaad, M. Kamal, S.I. Alqasoumi & M.E. Zain (2018) *Saudi Pharm J.*26: 279–285

3. Nelson V.K., N. K. Sahoo, M. Sahu H.H. Sudhan, C.P. Pullaiah & K.S. Muralikrishna (2020) *BMC Complement. Med.*20: 355: 1-8

4. Takdhat P.L. & A.V. Chandewar (2021) *J. emerg. technol. innov. res*. 8(12): b732-b746

5. Ashraf V.K., V.K. Kalaichelvan, V.V. Venkatachalam & R. Ragunathan. (2021) *Future J. Pharm. Sci.* 7: 50
6. Muniyandi K., E.George, V. Mudili, N.K Kalagatur, A.J. Anthuvan, K. Kadirvelu, P. Thangaraj & G. Natarajan (2017) *Agric. Nat. Resour.*51: 63-73
7. Greenwell M. & P.K.S.M. Rahman (2015) *Int J Pharm Sci Res*6(10): 4103–4112
8.Attar R., S. Tabassum, S. Fayyaz, M. S. Ahmad, D. R. Nogueira , I. Yaylim , O. Timirci-Kahraman , O. Kucukhuseyin , C. Cacina , A. A. Farooqi & M. Ismail (2015). *Cell Mol Biol*. 61 : 62– 68.
9. Kinghorn A.D, Y-W.Chin & S.M. Swanson (2009)*Curr Opin Drug Discov Devel.* 12: 189-196.
10. Al-Saraireh Y.M., A.M.M Youssef, A.Z. Alsarayreh,. T.A. Al Hujran, S. Al-Sarayreh, J.M. Al-Shuneigat & H.M.Alrawashdeh (2021) *J. Pharm. Pharmacogn. Res.*9(1): 13-23
11.Sharifi-Rad J, A. Ozleyen, T.B.Tumer, C.O.Adetunji, N.E. Omari, A.Balahbib, Y.Taheri A .Bouyahya, M. Martorell, N.Martins & W. C. Cho (2019) *Biomolecules*9:679:1-52
12.Marilena M, P. S. Eliezer, M. D. Jorge & P. D. Juceni (2012) *Rev. bras. farmacogn.*22(3):682-713
13.Sonia N.S. (2020) *J. pharmacogn. phytochem*. 9(1): 2118-2122
14.Mofiz U.K & S. Hossain(2015) *J. pharmacogn. Phytochem*. 4(2): 05-07
15.Kamta P.N., S. Naskar & N.R Beck(2021). *Research J. Pharm. and Tech.* 14(1): 369-372
16.Chandira M.& B. Jayakar(2010) *Int. J. Pharm. Sci. Rev. Res*. 3(1): 101-10.
17.Pandey A., P.P.Gupta & V.K. Lal(2013) *J Basic Clin Physiol Pharmacol*. 24(1): 35-9.
18.Kottai M.A., G. Alagumanivasagam, K. D. Satheesh & R. Manavalan(2011)*Res. J. Pharm. Biol. Chem. Sci*. 2(3):183-91.
19. Vasagam G.A, A.K.Muthu & R. Manavalan(2010) *Int. J. Pharma Bio Sci*. 1(2) :1-5
20. Jain V., S.K. Verma & S.S. Katewa(2011) *Indian J. Tradit. Knowl.*10 (4): 617-623
21. Khandelwal, K. R., (2006). *"Practical Pharmacognosy-Techniques and Experiments",* Sixth edition, Nirali Publication, Pune, India, pp. 149-156.
22.Antoney J., A. J. D. Britto, P. Abida & L.S.T. Raj(2016) *Adv. cytol. pathol*. 1(1):6-8
23. Shivpuje P, R. Ammanangi, K. Bhat, S. Katti(2015) *J. Contemp. Dent. Pract.*16(9):709-714