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# Cytotoxic potency in leaf ethanolic extract of *Glycosmis pentaphylla* (Retz.) DC.

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## Abstract

The plant *Glycosmis pentaphylla*, belongining to the family Rutaceae, were reported to possess several pharmacological properties due to the vast array of secondary metabolites. The present investigation was carried out to illustrate the cytotoxic properties of leaf extract on tumor cell lines. The leaf powder was subjected to successive extraction using solvents based on their polarity. From which, the ethanol extract showed more number of compounds, hence this was subjected to cytotoxicity studies. The cell viability was assessed by trypan blue exclusion method. The results revealed potent cytotoxicity for the extract, with $IC_{50}$ value 78.39$\mu$g/ml Dalton's Lymphoma Ascites cell lines. Thus, the cytotoxic properties of leaf extract could be utilized against various human cancer cell lines to reduce and eradicate tumor growth.

**Keywords**: *Glycosmis pentaphylla*, *in vitro* cytotoxicity, DLA cell line, $IC_{50}$ values.

## Introduction

Use of plants as anticancer agents began with folk medicine, and has been incorporated into ayurvedic and allopathic systems over the years (Valko *et al., 2006*). The use of medicinal plant extracts for the treatment of various ailments is a traditional practice and this has greatly increased in recent years (Khakdan *et al.,* 2013). Herbal anti-cancer compounds are unique in their feature of having anti-oxidant and immune-stimulant activity, preventing cancer growth indirectly along with a direct cytotoxic effect towards malignant and other apoptotic cells (Adhvaryu *et al.,* 2008). A wide variety of natural compounds in traditional medicinal plants appeared to possess significant cytotoxic as well as chemo preventive activities. Extracts of *Curcuma longa*, *Desmodium triangulare, Drosera indica*, *Ocimum sanctum*, *Orthosiphon thymiflorus, Tinospora cordifolia*, *Zingiber officinale*, *etc.* possessed significant antitumor potential against tumor cell lines Dalton's Lymphoma Ascites, Ehrlish Ascites Carcinoma and Human Skin Carcinoma cell lines (Asirvatham and Christina 2013, Jayaseelan *et al., 2014*, Hanafy, 2009, Sini *et al., 2012*). Cancer is one of the most life-threatening diseases with more than 100 different types. Due to lack of effective drugs, expensive cost of chemotherapeutic agents and their side effects, cancer can be a cause of death (Khakdan *et al.,* 2013). For a long time, plants have been used in the treatment of cancer which contain many effective anticancer agents (George *et al.,* 2010). This forms the basis for new drug discovery based on traditional plant usage. Currently, over 50% of drugs used in clinical trials for anticancer activity were isolated from natural sources or are related to them (Mohammad *et al.,* 2012, Newman *et al.,* 2007). Cancer is the prominent origin of death in efficiently developed countries and second largest death in developing countries. It is estimated that new cases of cancer will rise approximately 25% in each decade, aiming 24 million new cases per year in the year 2050 (Schwartsmann *et al.,* 2012). The current threat linked with presently existing drugs comprises selectivity, toxicity, resistance, and development of a secondary malignancy.

The downsides of these anticancer agents have encouraged the examination of novel, competent and well resistant drugs against cancer, as natural products, mainly from plants. Various signalling pathways in cancer cells are being regulated by plant-derived agents. It is also believed that plant constituents can restrain multiple signalling pathways concurrently; therefore, they can be very active in preventing uncontrolled cell propagation of cancer cells, which have numerous survival approaches. Naturally herbs are therapeutic sources, whereas chemotherapy and cytotoxic drugs are inherently destructive. The *in vitro* cytotoxicity assays determine the response of normal and cancer cell lines of human origin to therapeutic agents that are critical for the identification and pre-clinical evaluation of new potent anti-cancer drugs.

*Glycosmis pentaphylla,* known as a tooth brush tree is a member of the citrus family Rutaceae and is commonly known as orange berry and gin berry. The word *Glycosmis* is derived from Greek where "*Glykys*" means sweet and "*Osme"* means smell; alluding to the sweet scented flowers. The family Rutaceae contains about 100 genera and 800 species of herbs and *G. pentaphylla* is a shrub or a small trees. (Wealth of India, 2003). It is distributed in Bangladesh, India, Malaysia, Southern China to Philippines, and Australia (Wang *et al.,* 2006). Members belonging to Rutaceae are known to be a rich in active secondary metabolites. Even though, they are mainly used in the treatment of liver failure and cardiac arrhythmia, their reported efficacy of inducing apoptosis in tumor cells is noteworthy. The plant is known to possess a number of biological activities including antimycotic, antiviral, molluscicidal, antimalarial, teratogenic and cytotoxic properties (Ariful *et al.,* 2010, Balachandran *et al.,* 2008, Ambasta *et al.,* 2000). This plant is known to contain an acridone alkaloid arborinine, which possess anticancer properties. Literatures are available on cytotoxic activity of the genus *Glycosmis*. However, *G. pentaphylla* is yet to be subjected for its cytotoxic tests to ascertain its cytotoxic activities with potential clinical application.

## Materials and Methods

### Plant material

Fresh leaves of *Glycosmis pentaphylla* was collected from Thodupuzha (9.8959$^0$ N, 76.7184$^0$ S) during the month of April, 2019. The botanical identities were verified by the Curator, Department of Botany, University of Kerala, taxonomical features were critically studied and confirmed with the Flora of the Presidency of Madras (Gamble, 1918) and with other relevant available literatures. A voucher specimen was maintained (KUBH-6043) at the Herbarium, Department of Botany, University of Kerala.

### Preparation of Plant Extract

Extracted 500 mg of leaves for three times (10 min for one extraction), with 5 ml each of the five extractants. The extractants were chosen on the basis of their polarity, relatively low boiling points as well as their ability to evaporate. The extractants n-hexane (0.10), ethyl acetate (4.4), acetone (5.1), ethanol (6.5) and water (10.2) were selected based on the polarity according to Snyder and Kirkland (1979). Preliminary phytochemical analysis were conducted, from which the ethanolic extract possessed more phytoconstituents. Hence, this was subjected to cytotoxicity studies.

### *in vitro* cytotoxicity assay

The crude extract of *G. pentaphylla* was subjected for *in vitro* cytotoxicity assay using Dalton's Lymphoma Ascites cells (DLA). The tumour cells aspired from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by trypan blue exclusion method. Viable cell suspension (1X10 $^6$ cells in 0.1ml) was added to tubes containing various concentration of the test compounds and the volume was made up to 1 ml using phosphate buffered saline (PBS). Control tube contained only cell suspension. These assay mixture were incubated for 3 hour at 37$^0$ C. Further cell suspension was mixed with 0.1 ml of 1% trypan blue and kept for 2-3 minutes and loaded on a haemocytometer. Dead cells take up the blue colour of trypan blue while live cells do not take up the dye. The number of stained and unstained cells were counted separately. The percentage cytotoxicity was calculated using the following formula;

$$\% \text{ of cytotoxicity} = \frac{Number\ of\ dead\ cells}{Total\ number\ of\ cells} \times 100$$

### Calculations of 50% inhibitory concentrations ($IC_{50}$)

The half maximal inhibitory concentration ($IC_{50}$) 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 is needed to inhibit a given biological process by half. In other words, it is the half maximal (50 %) inhibitory concentration (IC) of a substance (50

% IC, or $IC_{50}$). Sometimes, it is also converted to the $pIC_{50}$ scale (-log $IC_{50}$), in which higher values indicate exponentially greater potency. According to the Food and Drug Administration (FDA), $IC_{50}$ represents the concentration of a drug that is required for 50 % inhibition *in vitro*. The concentration of the fractions that was required to scavenge 50 % of the radicals was calculated by using the percentage scavenging activities versus the concentration of extract using nonlinear regression analysis (curve fit) at five different concentrations of the extract and standard, as well. In a similar way, half maximal lethal concentration ($LC_{50}$) was also calculated.

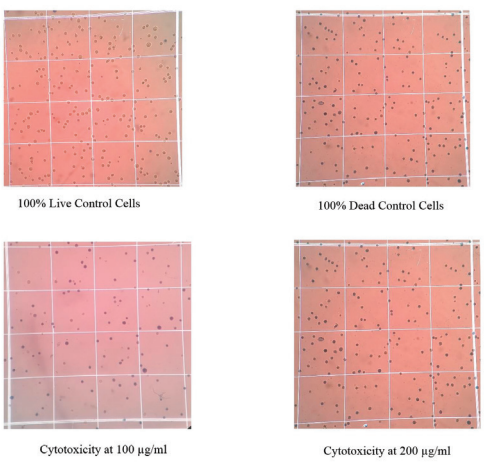
## Results and Discussion

Cancer is the prominent origin of death in efficiently developed countries and second largest death in developing countries. It is estimated that new cases of cancer will rise approximately 25% in each decade, aiming 24 million new cases per year in the year 2050 (Schwartsmann *et al.,* 2012). The current threat linked with presently existing drugs comprises selectivity, toxicity, resistance, and development of a secondary malignancy. The downsides of these anticancer agents have encouraged the examination of novel, competent and well resistant drugs against cancer, as natural products, mainly from plants. Various signalling pathways in cancer cells are being regulated by plant-derived agents. It is also believed that plant constituents can restrain multiple signalling pathways concurrently; therefore, they can be very active in preventing uncontrolled cell propagation of cancer cells, which have numerous survival approaches. Naturally herbs are therapeutic sources, whereas chemotherapy and cytotoxic drugs are inherently destructive. The *in vitro* cytotoxicity assays determine the response of normal and cancer cell lines of human origin to therapeutic agents that are critical for the identification and pre-clinical evaluation of new potent anti-cancer drugs. Keeping this in mind, the present investigations have been carried out to screen the cytotoxic potential of leaf ethanolic extract in RAW 264.7 cell lines. Cytotoxicity assays normally involve short-term exposure of cultured cells to test substances in order to predict potential toxicity. It also provides insight towards the carcinogenic and genotoxic dispositions of herb-derived compounds and extracts. Among various parameters to detect cytotoxicity, the trypan blue test is often used to measure cell membrane integrity, as it forms a functional barrier around the cells which regulate and interchange molecules into and out of cells. The evaluation of cytotoxic potential of crude extract revealed the ability of extract in limiting the growth of toxic cells. The results are summarized in Table 1. The $IC_{50}$ of *G. pentaphylla* extracts on RAW 264.7 cell line was also calculated for the extract at concentrations ranging from 10 to 200 $\mu g$/ml. $IC_{50}$ indicates the lowest concentration of plant extracts that inhibits 50 % of cells. The leaf ethanol extract had high $IC_{50}$ of 78.39$\mu$g/ml. The results revealed that the as the concentration of the extract increased, the percentage of cytotoxicity also increased in a dose dependent manner (Fig 1). Extracts with high $LC_{50}$ are preferable to work with, because of their lower toxicity effects on the host cells.

**Table 1: *in vitro* cytotoxicity assay of *Glycosmis pentaphylla***

| Sl.No | Concentration (µg/ml) | % Cytotoxicity |
|---|---|---|
| 1 | 10 | 8.4 |
| 2 | 20 | 16.2 |
| 3 | 50 | 34.6 |
| 4 | 100 | 86.5 |
| 5 | 200 | 98.4 |

**Figure 1: *in vitro* cytotoxicity assay in leaf ethanolic extract of *Glycosmis pentaphylla***




The results revealed that the as the concentration of the extract increased, the percentage of cytotoxicity also increased in a dose dependent manner Extracts with high $LC_{50}$ are preferable to work with, because of their lower toxicity effects on the host cells. The presence of key phytoconstituents in ethanolic extracts may be the main reason of their cytotoxicity against the cell

line used. This extract have shown the existence of major phytoconstituents viz: alkaloids, flavonoids, and terpenoids which may be connected for their influence on cytotoxicity. The foundation of alkaloids with anticancer possibilities is very broad. Exploration of alkaloids such as piperine is generally focused on cancer prevention (Selvendiran *et al.,* 2003; Manoharan *et al.,* 2010). The role of flavonoids in cancer prevention indicate that flavonoids have important effects on cancer chemoprevention and chemotherapy (Ren, 2003). Terpenoids have been found to be valuable in the restriction and therapy of cancer (Rabi *et al.,* 2009). An enormous number of triterpenoids have been presented to suppress the progression of a number of cancer cells without employing any toxicity in normal cells (Setzer *et al.,* 2006; Petronelli *et al.,* 2009). By considering the effects of all these phytoconstituents, it is possible that ethanolic leaf extracts of *G. pentaphylla* might have affected the cancer cell lines because of rich presence, and stability of these phytoconstituents, because the qualitative analysis of these extracts has been resulted in the existence of these secondary metabolites.

## Conclusion

The present study revealed that crude ethanolic extract of *G. pentaphylla* leaves displayed strong cytotoxic potential against DLA tumor cell lines. The results revealed a dose dependent cytotoxic potential. Ethanol can be considered as an effective solvent in extracting many of the active biomolecules. Hence, it can be effectively utilized for the successful extraction of various biomolecules useful against cancer cell lines as well as in the treatment of various ailments, thereby helping in the development of valuable drugs.

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## References

Adhvaryu MR, Reddy N, Parabia MH. Antitumor activity of four ayurvedic herbs in dalton lymphoma ascites bearing mice and their short-term *in vitro* cytotoxicity on DLA-Cell-Line. Afr J Tradit Complementary Altern Med 2008;5:409-18.

Ambasta SP. (2000). The useful plants of India. *Published by National Institute of Science and Communication. New Delhi: 4th edition*, 380-381.

Anonymous, A. (2003). The wealth of India: A dictionary of Indian raw materials and industrial products.

Ariful HM, Romeo M, Rohimul F, Kishore KT, Azmal IH, Bulbul A. (2010). Abstract A101: Ethnomedicinal uses of some medicinal plants for prevention against all forms of cancer by the traditional healers in Gazipur district of Bangladesh. *AACR International conference on frontiers in cancer prevention research. Cancer Prev Res. Vol 3*.

Asirvatham R, Christina AJM. Anticancer activity of *Drosera indica* L. on dalton's lymphoma ascites (DLA) bearing mice. J Intercultural Ethnopharmacol 2013;2:9-14.

Balachandran I, Udayan PS, Harinarayanan MK, Tushar KV. (2008). Some common plants used by kurichiar tribes of tirunelli forest, Wayanad district, Kerala in medicine and other traditional uses. *Indian J Tradit Knowl. 07*, 250-255.

Gamble JS (1918). The Flora of Presidency of Madras. Vol. 2, *Botanical Survey of India, Calcutta,* 1215-1227**.**

George S, Bhalerao SV, Lidstone EA. Cytotoxicity screening of Bangladeshi medicinal plant extracts on pancreatic cancer cells. BMC Comp Alt Med 2010;10(1):52-5.

Hanafy ZE. Ginger extract antimutagens as cancer chemopreventive agent against ehrlich ascites carcinoma. Acad J Cancer Res 2009;2:61-7.

Jayaseelan RS, Vijayan FP, Mathesvaran M, Suresh V, Padikkala J. Cytotoxic and antitumor activity of methanolic extracts *Desmodium triangulare* (retz) merr. Root Int J Pharm Pharm Sci 2012;4:540-2.

Khakdan F, Piri K. In vitro cytotoxic activity of aqueous root extract of *Althea kurdica* against endothelial human bone marrow cells (line k562) and human lymphocytes. Pharm Life Sci 2013;2(6):23-9.

Manoharan, S., Muneeswaran, M., & Baskaran, N. (2010). Chemopreventive efficacy of berberine in 7, 12-dimethylbenz [a] anthracene (DMBA) induced skin carcinogenesis in Swiss albino mice. *International Journal of Research in Pharmaceutical Sciences*, *1*(4), 521-529.

Mohammad S, Kashani HH, Azarbad Z. Capparis spinosa L. Propagation and medicinal uses. Life Sci J 2012;9(4):684-6.

Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. J Nat Pro 2007;70:461-77.

Petronelli, A., Pannitteri, G., & Testa, U. (2009). Triterpenoids as new promising anticancer drugs. *Anti-cancer drugs*, *20*(10), 880-892.

Rabi, T., & Bishayee, A. (2009). Terpenoids and breast cancer chemoprevention. *Breast cancer research and treatment*, *115*(2), 223-239.

Ren, W., Qiao, Z., Wang, H., Zhu, L., & Zhang, L. (2003). Flavonoids: promising anticancer agents. *Medicinal research reviews*, *23*(4), 519-534.

Schwartsmann, G., de Almeida Sassi, F., Lunardi Brunetto, A., Roesler, R., & Abujamra, A. L. (2012). Glioma revisited: from neurogenesis and cancer stem cells to the epigenetic regulation of the niche. *Journal of oncology*, *2012*.

Selvendiran, K., Singh, J. P. V., Krishnan, K. B., & Sakthisekaran, D. (2003). Cytoprotective effect of piperine against benzo [a] pyrene induced lung cancer with reference to lipid peroxidation and antioxidant system in Swiss albino mice. *Fitoterapia*, *74*(1-2), 109-115.

Setzer, W. N., Schmidt, J. M., Noletto, J. A., & Vogler, B. (2006). Leaf oil compositions and bioactivities of abaco bush medicines. *Pharmacologyonline*, *3*, 794-802.

Sini KR, Haribabu Y, Sajith MS, Sreekumar SK. *The in-vitro* cytotoxic activity of (*Orthosiphon thymiflorus* Roth.) sleensen leaf extract against dalton lymphoma ascites cell line. J Chem Pharm Res 2012;4:917-21.

Snyder, H. L., & Kirkland, J. J. (1979). Chapter 4". *Image quality: Measures and visual performances" In Flat*.

Valko V, Fickova M, Pravdov E, Nagya M, Grancai D, Czigle S. Cytotoxicity of water extracts from leaves and branches of *Philadelphus coronarius* L. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2006;150:71-3.

Wang, J., Di, Y., Yang, X., Li, S., Wang, Y., & Hao, X. (2006). Hydroquinone diglycoside acyl esters from the stems of *Glycosmis pentaphylla*. *Phytochemistry*, *67*(5), 486-491.

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