Int J Pharm Bio Sci 2016 April; 7(2): (B) 204 - 211

**Original Research Article** **Bio pharmaceutics**

**International Journal of Pharma and Bio Sciences** **ISSN 0975-6299**

# EXTRACTION OF VINORELBINE FROM LEAVES *OF CATHARANTHUS ROSEUS* AND ITS APPLICATION IN TREATING BREAST CANCER USING *IN VIVO* MOUSE MODELS

**GIREESH KAMATH H[1*], DEECARAMAN[1] AND RAMESH KV[2]**

*[1]Dr. M. G. R. Educational and Research institute, Maduravoyal, Chennai-600095*
*[2] Center for Postgraduate Studies, Jain University, 18 / 3, 3rd Block, 9th Main, Jayanagar, Bangalore - 560011*

## ABSTRACT

Extracts from the leaves of *Catharanthus roseus* were collected using methanol. Three individual alkaloids were fruitfully separated by TLC, purified using column chromatography and one of them was successfully identified as vinorelbine. Nude mice xenografted with breast cancer cell line MDAMB231 were treated through vinorelbine (30 mg/kg body weight, i.p.). Mice responded to the treatment and showed a significant decrease in the tumor volume in comparison to mice of the control growth. Results shows that the plant extracted vinorelbine may be helpful for treating the breast cancer.

**KEY WORDS:** **Breast cancer, plant extract, nude mice, xenograft, Vinorelbine, tumor volume.**

**GIREESH KAMATH H**
**Dr. M. G. R. Educational and Research Institute, Maduravoyal, Chennai-600095**

## INTRODUCTION

Traditional use of herbal medicine has been a major source for the discovery of novel anti-cancer agents. Specific biological activities of these products may be because of their highly complex molecular structures coupled with different chiral centres in relation to small molecules[1]. In a recent review, it has been shown that nearly 60 % of the new and approved anticancer drugs were either natural products or their derivates. Further, these drug products contain pharmacophores, either derived from natural sources or from modified natural products attached to targeting systems[2]. Plant-derived compounds with anti-tumor activity comprise camptothecin derivatives, topotecan and irinotecan, etoposide derived from epipodophyllotoxin along with paclitaxel, and vinca plants exhibits potential anti-tumor properties amidst several important anti-cancer plant extracts[3,4]. There are at least 86 alkaloids extracted from vinca genus. Among these, vincristine, vindesine and vinflunine extracted from *Vinca rosea* are used to treat leukemia, lymphomas and childhood cancers, as well as several other types of cancer including some non-cancerous conditions[5,6]. Vinorelbine, one of the plant extracts from *Vinca rosea*, has been reported to show activity against breast cancer protein transferase. This compound is obtained by hemisynthesis, which makes the molecule lipophilic compared to others[7]. *In silico* docking studies strongly suggest that vinorelbine to be a better alternative to the existing drug tamoxifen[8]. Organic solvents are generally used to extract various natural derivatives from the plants, which is used either singly or as a mixture. For instance, removal of surface waxes as well as other hydrophobic surface chemicals from plant tissues is achieved by dipping *Vinca* leaves in chloroform[9]. In another experiment, vinblastine in the sulfate form and vinca alkaloid as dimethyl sulfonate salt was isolated using acetonitrile solvent[10]. Using solvent mixture methanol-acetonitrile (75:25, v/v), Barthe *et al*[11] (2002) were successful in separating 11 alkaloids from vinca plants. Secondary extracts from the young leaves of *Avicennia marina* were extracted using different solvents with the help of Soxhlet apparatus. Thin Layer Chromatography (TLC) was used subsequently to separate the leaf extracts components[12]. Column chromatography is also used as one of the methods for purifying phytoextracts. Two phytoextracts from the seeds of *Mimusops elengi* identified using ethyl acetate were purified by column chromatography[13]. *In vitro* cytotoxic activity of various plant extracts have been used for the purpose of selecting extracts exhibiting potential therapeutic properties[14-17]. *In vitro* cytotoxicity of *Centella asiatica*, *Curcuma longa* and *Strobilanthes crispus* extracts were conducted with three kidney cell lines obtained from African green monkey, baby hamster and rabbit using MTT reduction assay. The results revealed *Centella asiatica* was the least toxic to all these cell lines tested followed by *Strobilanthes crispus* and *Curcuma longa*[14]. Among the total of 14 wild angiosperms collected from Saudi Arabia, cytotoxic activity exhibited by the extract of *Lavandula dentata* showed promising potential anticancer agent having good anti-proliferative as well as apoptotic activity[15]. There are several studies which show vinca based alkaloids extracted from *Catharanthus roseus* and their synthetic derivatives display good anti-tumor properties[18-22]. In a recent study, cytotoxicity of Vinorelbine was tested along with other neoplastic drugs (Geftinib, Cisplatin, 5-FU and Gemcitabine) using cervical cancer cell lines. From this study, cisplatin was found to be the most toxic drug ($LC_{50}$ = 13μM), while vinorelbine was least toxic ($LC_{50}$ = 48μM)[22]. Animal models of cancer in mice offer additional choice of experiments to determine the causes and treatments for malignancy. Further, these models assist the investigators in observing and manipulating a complex disease process in a manner impossible to execute in patients. In the recent past, numbers of studies have been conducted using animal models to understand the pathogenesis and response to treatment of many of the cancer and other diseases[23-27]. The plant extracts were tested initially for their cytotoxicity under *in vitro* condition followed by *in vivo* compatible tumor models in order to know its effectiveness against cancer[28-31]. For instance, combination treatment of vinorelbine with fulvestrant and other cytotoxic agents (doxorubicin, paclitaxel, docetaxel and 5-fluorouracil) has a synergistic effect in estrogen receptor-positive breast cancer. The outcome of the study is based on both *in vitro* as well as *in vivo* investigations[28]. Based on *in vitro* and later by nude mice models, a recombinant humanized anti-insulin-like growth factor receptor type I antibody (h7C10) enhanced the antitumor activity of vinorelbine and anti-epidermal growth factor receptor therapy against human cancer xenografts[30]. Several studies have revealed that different alkaloids present in *Catharanthus roseus* extracts are showing numerable therapeutic uses. Currently, vincristine, vindesine, vinflunine, vinblastine and vinorelbine are few alkaloids extracted from vinca which have extensive pharmaceutical applications. Though vinorelbine is well known for its anti-cancer properties[21, 22], the compound has not been explored for therapeutic purpose in its natural form, instead the molecule is synthesized which is expensive. Alternatively, compound extracted in its natural form may be safer as well as cost effective. In this context, an attempt is made in the present investigation to study vinorelbine, a natural plant extract from *Catharanthus roseus* for its anti- breast cancer properties using *in silico* and *in vivo* approaches.

## MATERIALS AND METHODS

The *Catharanthus roseus* (periwinkle) plants were procured from nursery of Horticulture department, Lalbagh, Bengaluru. The plant was identified in National Ayurveda Dietetics Research Institute, Bangalore as *Catharanthus roseus* (L.) G. Don, (authentication/S.M.P.U./N.A.D.R.I./BNG/201516) Extraction and purification of vinorelbine from *Catharanthus roseus* was undertaken. Followed by this extract was tested in animal models to prove the efficacy.

***Extraction***

Dry leaf powder of *Catharanthus roseus* mixed with methanol was kept on shaker. The resulting filtrate on filter paper was dried and was subsequently extracted by Soxhlet extractor using methanol at 70-80 °C [34]. The pure methanol extract retained in a round bottom flask, was stored in refrigerated condition. The dried extract was placed in acidified distilled water and heated to about 50 °C for 24 hrs. The solution was filtered through filter paper. The filtrate compound was defatted for 24 hours after adding hexane. The defatted products present in the solvent was decanted and discarded, leaving behind alkaloid rich residue. The residue after treating with strong alkaline solution was kept in warm conditions with constant stirring for 20 minutes. The alkaloid upon migration in hexane was left for 24 hrs for the formation of thick emulsion. Sodium hydroxide solution was later added to break the emulsion that was formed; and solvent layer was subsequently separated from the solution. The solvent was finally evaporated to yield alkaloids free from base[32].

***Thin layer chromatography* and column chromatography**

Silica coated plates were air dried at room temperature for one hour and dried at 80 °C in an oven for 30 minutes. Alkaloid samples were spotted onto the TLC plate that was completely immersed inside the solvent, which was allowed to evaporate completely. Thin layer chromatography (TLC) was performed for differentiating the alkaloids. Plant extracts obtained by Soxhlet extraction were further purified using column chromatography technique. Chemical characterization done through IR and $^{1H}$NMR spectral analyses confirmed one of the isolated compounds was vinorelbine (data not presented).

***Animal experiments***

*In vivo* experiment was carried out using female mice (*Mus musculus*), strain Hsd: Athymic Nude-Foxn1nu (source; Harlan) of 5-6 weeks. Experiment was carried out per CPCSEA approved Institute of Animal Ethics committee protocol number SYNGENE/IAEC/537/08-2014. Body weight of the animals varied between 20-22 g. Animals were dosed with vinorelbine at the rate 30 mg / kg body weight through intra-peritoneal route once weekly, for 4 weeks. Concurrent control group was also kept. Eight animals xenografted with breast cancer cell line MDAMB231 were used for both the groups. Each animal was carrying $15x10^6$ cells present in right abdominal flank subcutaneously. At study initiation tumor volume was approximately, 150 $mm^3$. All the animals were observed for the clinical signs, once daily. The animals were provided with feed and water, *ad libitum*, throughout the treatment period. The tumor volume was measured once every three days along with animal body weight[26].

Tumor volume (TV) was calculated as follows

$$TV\ (mm^3) = \frac{\text{Length of tumor} * \text{width of tumor}^2}{2}$$

Tumor growth inhibition (TGI) was calculated as follows

$$TGI\ \% = \frac{TV\ \text{of treatment}\ (mm^3) - TV\ \text{of treatment}\ (mm^3)}{TV\ \text{of control}\ (mm^3)} X100$$

Tumor growth inhibition was statistically analyzed using students-t test[33].

## RESULTS

Solvent extract of dried leaf powder from *Catharanthus roseus* when separated by TLC displayed presence of three distinct spots (Fig 1) and was subsequently purified by column chromatography. The isolated vinorelbine, which was confirmed using IR and $^{1H}$NMR spectral analyses, was taken for *in vivo* studies. Average tumor volume observed was compared between control and vinorelbine treated animals for 30 day treatment period (Fig 2). Data when subjected to Students-t test suggests significant reduction in tumor volume from 18$^{th}$ day onwards in vinorelbine treated group in relation to control group. This is further supported by the fact that the extent of tumor growth inhibition reached a maximum of 80 % in nude mice treated with vinorelbine at the end of the treatment (Fig 3). While body weights of vinorelbine treated animals were lower, it was relatively higher in untreated animals. Overall, there was 5 % reduction in mean body weight of vinorelbine treated animals and 8 % increase in the control group at the end of treatment (Fig 4).

## DISCUSSION

Three distinct alkaloids were successfully separated from the leaves of *Catharanthus roseus* using TLC and were subsequently purified with the help of column

chromatography. TLC and column chromatography are widely used techniques for separation of compounds from plant extracts. For instance, Abeysinghe *et al* (2011)[12] used TLC and column chromatography methods for extracting secondary metabolites from *Avicennia marina* plant to be tested as antibacterial compound. In the present study, among various compounds that were extracted, presence of vinorelbine was confirmed by performing IR and $^{1H}$NMR spectral analyses. Hazra *et al* (2007)[13] used similar techniques for identifying two antibacterial compounds from seeds of *Mimusops elengi. In vivo* studies conclusively prove that the animals xenografted with breast cancer cell lines and treated with vinorelbine purified from plant extract started showing recovery signs after 18 days of treatment. The average tumor volume as well as the weight of the vinorelbine treated animals reduced constantly. Solowey *et al*, 2014[34], examined the effects of three different whole plant extracts on human tumor cells. Plant extract from *Urtica membranacea* showed strong anticancer capabilities since it inhibited actual tumor progression in a breast adenocarcinoma mouse model. Vinorelbine extracted from Vinca plant in the present study may exert their anticancer actions by disturbing polymerization of tubulins. This is in agreement with the observations made by Yu *et al* (2004) [35]. Further, Nirmala *et al* (2011) [36], in a recent review have also reported mechanism of action for Vinca alkaloids, wherein these compounds inhibit cell proliferation by affecting microtubular dynamics during mitosis and there by causing a characteristic block during mitosis leading to apoptosis. Paganini-Hill and Clark (2000)[37] have reported that tamoxifen, currently available drug for breast cancer, may adversely effect cognition in the patients. Our earlier *in silico* studies have clearly shown vinorelbine having better docking energy than tamoxifen (Kamath *et al*, 2014)[8], thereby suggesting this compound, if extracted in its natural form could be a better choice for breast cancer therapy having lesser side effects.

**Figure 1**
***Separation of alkaloids from the solvent extract of dried leaf powder from Catharanthus roseus using thin layer chromatography***

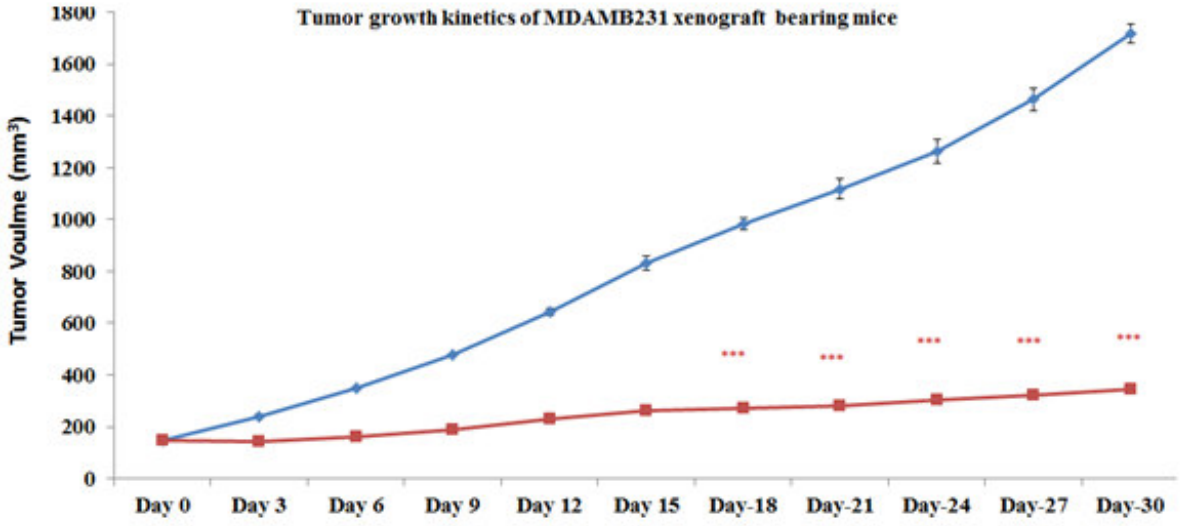


**Figure 2**

***Tumor growth volume in nude mice bearing MDAMB 231 xenograft treated with vinorelbine extracted from the dried leaf powder of Catharanthus roseus. [*** refers to significant in student-t test ($p < 0.001$)]. Nude mice xenografted with MDAMB231 breast cancer cell lines were dosed with vinorelbine @ 30 mg / kg body weight.***

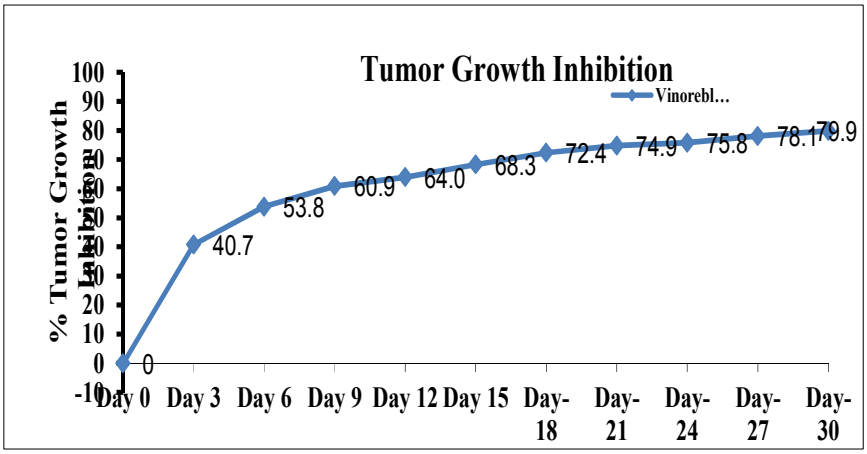


**Figure 3**

***Tumor growth inhibition curve in nude mice treated with vinorelbine extracted from the dried leaf powder of Catharanthus roseus. Nude mice xenografted with MDAMB231 breast cancer cell lines were dosed with vinorelbine @ 30 mg / kg body weight.***

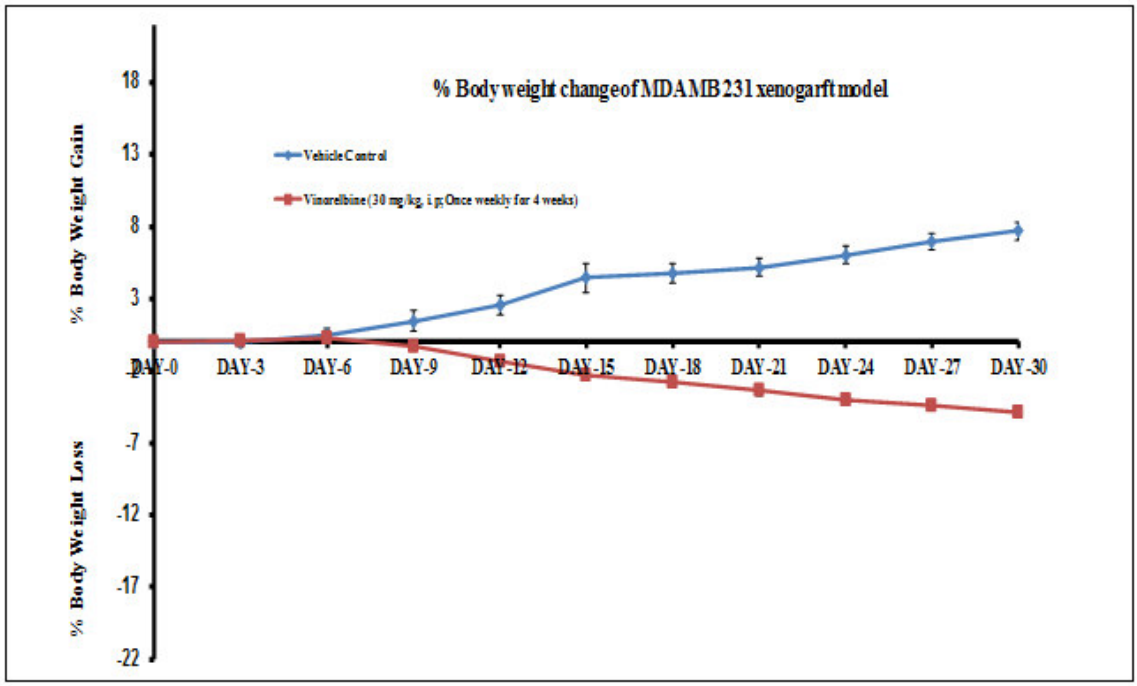


**Figure 4**

***Per cent body weight change in nude mice treated with vinorelbine extracted from dried leaf powder of Catharanthus roseus. Nude mice xenografted with MDAMB231 breast cancer cell lines were dosed with vinorelbine @ 30 mg / kg body weight***

## CONCLUSION

Vinorelbine from leaf extracts of *Catharanthus roseus* were treated against breast cancer cell xenografted nude mice resulted in successfully controlling the breast cancer to a significant lower level. This may not only be helpful in bringing down the cost of the drug, but also helpful in bringing down side effects of synthetic drugs present in the current market.

## ACKOWLEDGEMENT

Honest thanks Syngene International Limited for providing the opportunity to carry forward the work and also, to friend Guruprasad for providing support in product extraction as well as purification.

## REFERENCES

1. Gonzales GF and Valerio LG Jr. Medicinal plants from Peru: a review of plants as potential agents against cancer. Anticancer Agents Med Chem. 2006; 6(5):429 – 444.
2. Kaefer CM and Milner JA. The role of herbs and spices in cancer prevention. J nutr Biochem. 2008; 19(6):347 – 361.
3. Gordon M. Cragg and David J. Newman. Perspective paper plants as a source of anti-cancer agents. Journal of Ethnopharmacology. 2005; 100:72 – 79.
4. Gordon M. Cragg and David J. Newman. Antineoplastic agents from natural sources: achievements and future directions. Expert Opin Investig Drugs. 2000;9(12): 2783 – 2797.
5. Jonathan Roepkea, Vonny Salima, Maggie Wua, Antje M. K. Thamma, Jun Muratab, Kerstin Plossc, *et al*. Vinca drug components accumulate exclusively in leaf exudates of Madagascar periwinkle. PNAS. 2010;107(34): 15287 – 15292.
6. Manfred Hesse. In: Meifsner W, editor. Alkaloids: nature's curse or blessing? Germany: Wiley-VCH.ISBN; 2002. p.7.
7. Marty M, Fumoleau P, Adenis A, Rousseau Y, Merrouche Y, Robinet G, Senac I and Puozzo. Coral vinorelbine pharmacokinetics and absolute bioavailability study in patients with solid tumors. Ann Oncol. 2001; 12(11):1643 – 1649.
8. Gireesh Kamath H, Deecaraman and Ramesh KV. Molecular docking studies of herbal ligands showing anti-tumor properties. Int J Pharm Bio Sci. 2015;6(2):1140 – 1154.
9. Samuels L, Kunst L and Jetter R. Sealing plant surfaces: cuticular wax formation by epidermal cells. Annu Rev Plant Biol. 2008; 59:683 – 707.
10. Olaf van Tellingen, Jos H. Beijnen, Willem J. Nooijen, and Auke. Plasma pharmacokinetics of vinblastine and the investigational vinca alkaloid N-(Deacetyl-O-4-vinblastoyl-23)-L-ethyl

isoleucinate in mice as determined by High-Performance Liquid Chromatography. Cancer Res. 1993; 53:2061 – 2065.
11. Barthe L, Ribet JP, Pélissou M, Degude MJ, Fahy J and Duflos A. Optimization of the separation of vinca alkaloids by nonaqueous capillary electrophoresis. J Chromatogr A. 2002; 968(1-2):241 – 250.
12. Abeysinghe Pushpa D and Weeraddana Chaminda De S. Screening of petroleum ether, chloroform, ethyl acetate, ethanol and water extracts of medicinal plant, *Avicennia marina* for antibacterial activity against antibiotic resistant bacteria species, *Staphylococcus* and *Proteus*. Journal of Pharmaceutical and Biomedical Sciences. 2011; 11:1 – 4.
13. Hazra KM, Roy RN, Sen SK and Laskar S. Isolation of antibacterial pentahydroxy flavones from the seeds of *Mimusops elengi* Linn. African Journal of Biotechnology. 2007;6(12): 1446 – 1449.
14. Hanisa H, Mohdazmi ML, Suhaila M and Hakim MN. Effects of *Centella asiatica* L., *Curcuma longa* L., and *Strobilanthes crispus* L. extracts on 3 kidney cell Lines: *in vitro* cytotoxicity analysis. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6:388 – 392.
15. Ali MA, Abul Farah M, Al-Hemaid FM and Abou-Tarboush FM. *In vitro* cytotoxicity screening of wild plant extracts from Saudi Arabia on human breast adenocarcinoma cells. Genetics and Molecular Research. 2014; 3(2): 3981 – 3990.
16. Soundararajan Vijayarathna and Sreenivasan Sasidharan. Cytotoxicity of methanol extracts of *Elaeis guineensis* on MCF-7 and vero cell lines. Asian Pac J Trop Biomed. 2012;2(10):826 – 829.
17. Shruti Bandopadhyaya, Mani Ramakrishnan Ramesh Puttalingaiah Thylur and Yogisha Shivanna. *In-vitro* evaluation of plant extracts against colorectal cancer using HCT 116 cell line. International Journal of Plant Science and Ecology. 2015; 1(3):107 – 112.
18. Culine S, Roch I, Pinguet F, Romieu G and Bressolle F. Combination paclitaxel and vinorelbine therapy: *in vitro* cytotoxic interactions and dose-escalation study in breast cancer patients previously exposed to anthracyclines. Int J Oncol. 1999; 14(5):999 – 1006.
19. Maryam Moudi, Rusea Go, Christina Yong Seok Yien and Mohd. Nazre. Vinca alkaloids. Int J Prev Med. 2013; 4(11):1231 – 1235.
20. Jean-Decoster C, Brichese L, Barret JM, Tollon Y, Kruczynski A, Hill BT and Wright M. Vinflunine, a new vinca alkaloid: cytotoxicity, cellular accumulation and action on the interphasic and mitotic microtubule cytoskeleton of PtK2 cells. Anticancer Drugs. 1999; 10(6):537 – 543.
21. Sevilcan Tuna, Meric A. Altinoz, Aykut Karasu, Ali Canbolat and Ayhan Bilir. Selective COX-2 inhibitor (rofecoxib) inhibits vinorelbine cytotoxicity in C6 glioma cells in vitro. The Open Pathology Journal. 2009;3:22 – 29.
22. Ahmed M and Jamil K. Cytotoxicity of neoplastic drugs gefitinib, cisplatin, 5-FU, gemcitabine and vinorelbine on human cervical cancer cells (HeLa). Biology and Medicine. 2011;3(5):60 – 71.
23. Frese KK and Tuveson DA. Maximizing mouse cancer models. Nat Rev Cancer. 2007;7:645 – 658.
24. Beverly A. Teicher. Tumor models for efficacy determination. Mol Cancer Ther. 2006;5(10): 2435 – 2443.
25. Barbara Szymanska, Urszula Wilczynska-Kalak, Min H. Kang, Natalia L. M. Liem Hernan Carol, *et al*. Pharmacokinetic Modeling of an induction regimen for *in vivo* combined testing of novel drugs against pediatric acute lymphoblastic leukemia xenografts. PLoS ONE. 2012; 7(3):1 – 14.
26. Joann CL Schuh. Trials, tribulations, and trends in tumor modeling in mice. Toxicologic Pathology. 2004;32:53 – 66.
27. Valerie J Poirier, Kristine E Burgess, William M Adams and David M Vail. Toxicity, dosage, and efficacy of vinorelbine (navelbine) in dogs with spontaneous neoplasia. J Vet Intern Med. 2004; 18:536 – 539.
28. Hirokuni Ikeda, Naruto Taira, Tomohiro Nogami, Kazuhiko Shien, Masanori Okada, Tadahiko Shien, *et al*. Combination treatment with fulvestrant and various cytotoxic agents (doxorubicin, paclitaxel, docetaxel, vinorelbine, and 5-fluorouracil) has a synergistic effect in estrogen receptor-positive breast cancer. Cancer Sci. 2011; 102:2038 – 2042.
29. Gui-Zhen Han, Zhi-Jian Liu, Kayoko Shimoi, and Bao Ting Zhu. Synergism between the Anticancer Actions of 2-Methoxyestradiol and Microtubule-Disrupting Agents in Human Breast Cancer. Cancer Res. 2005;65(2):387 – 393.
30. Goetsch L, Gonzalez A, Leger O, Beck A, Pauwels PJ, Haeuw JF, *et al*. A recombinant humanized anti-insulin-like growth factor receptor type I antibody (h7C10) enhances the antitumor activity of vinorelbine and anti-epidermal growth factor receptor therapy against human cancer xenografts. Int J Cancer. 2005; 113(2):316 – 328.
31. N Alami, J Paterson, S Belanger, S Juste, CK Grieshaber and B Leyland-Jones. Comparative analysis of xanafide cytotoxicity in breast cancer cell lines. British Journal of Cancer. 2007;97:58 – 64.
32. Soxhlet F. Dhtsanalytische Bestimmung des Milchfettes., Dingler's Polytechnisches Journal. 1879;232:461 – 465.
33. Blair R. Clifford and Higgins James J. A comparison of the power of Wilcoxon's rank-sum statistic to that of student's t statistic under various nonnormal distributions. Journal of Educational Statistics. 1980;5(4):309 – 335.

34. Elisha Solowey, Michal Lichtenstein, Sarah Sallon, Helena Paavilainen, Elaine Solowey, and Haya Lorberboum-Galski. Evaluating Medicinal Plants for Anticancer Activity. The Scientific World Journal. 2014;2014:1 – 12.
35. Jina Yu, Zhi-Jian Liu, Gui-Zhen Han, Anthony J. Lee and Bao Ting Zhu. Precipitous dose-response curves for the anticancer actions of microtubule-disrupting agents in human breast cancer cells: implications for high-dose regimen in anticancer chemotherapy. Med Hypotheses Res. 2004;1:267 – 274.
36. M. Joyce Nirmala, A. Samundeeswari and P. Deepa Sankar. Mini-Review natural plant resources in anti-cancer therapy-A review. Research in Plant Biology. 2011;1(3):01-14.
37. Paganini-Hill A and Clark LJ. Preliminary assessment of cognitive function in breast cancer patients treated with tamoxifen, Breast Cancer Research and Treatment. 2000;64(2): 165 –176.