Cytotoxic activity of *Macrosolen parasiticus* (L.) Danser on the growth of breast cancer cell line (MCF-7)

Vijay Kumar Sodde, Richard Lobo, Nimmy Kumar, Rajalekshmi Maheshwari, C. S. Shreedhara

Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka, India

Submitted: 31-10-2014 Revised: 10-12-2014 Published: 27-05-2015

**ABSTRACT**

**Background:** *Macrosolen parasiticus* (L.) Danser belonging to Loranthaceae (mistletoe family) is a parasitic plant that grows on different host plants such as mango, jack fruit, peepal, neem tree, etc. This study was aimed to investigate the anti-cancer activity of methanolic and aqueous extract of stem of *M. parasiticus*. **Objectives:** To investigate the *in vitro* cytotoxic potential of the methanolic and aqueous extracts from stems of *M. parasiticus* against MCF-7 breast cancer cells by brine shrimp lethality (BSL) bioassay, MTT assay and sulforhodamine B (SRB) assay. **Materials and Methods:** The extracts were tested in human breast cancer cell lines *in vitro* for percentage cytotoxicity, apoptosis by acridine orange/ethidium bromide staining, LD₅₀ and IC₅₀ values after treatment with *M. parasiticus* extracts. **Results:** In BSL bioassay, aqueous extract showed more significant (*P* < 0.01) cytotoxicity with LD₅₀ 82.79 ± 2.67 μg/mL as compared to methanolic extract with LD₅₀ 125 ± 3.04 μg/mL. The methanolic extract of *M. parasiticus* showed IC₅₀ 97.33 ± 3.75 μg/mL (MTT) (*P* < 0.05) and 94.58 ± 3.84 μg/mL (SRB) (*P* < 0.01) assays against MCF-7. The aqueous extract of *M. parasiticus* demonstrated higher activity with IC₅₀ 59.33 ± 3.3 μg/mL (MTT) (*P* < 0.01) and 51.9 ± 1.87 μg/mL (SRB) (*P* < 0.01) assays, after 48 h of exposure and thus showed significant dose-dependent cytotoxic activity. **Conclusion:** The finding demonstrated that both extracts of *M. parasiticus* showed significant cytotoxic activity, however aqueous extract demonstrated higher activity against MCF-7 breast cancer cells.

**Key words:** Apoptosis, breast cancer, cytotoxicity, *Macrosolen parasiticus* extracts

**INTRODUCTION**

Cancer is considered as one of the most terrified diseases and it’s a class of diseases or disorders characterized by uncontrolled cell proliferation and tissue invasion or metastasis of abnormal cell in the body.[3] breast cancer is the second leading cause of death in women[2] and in breast cancer bone, lungs, liver, chest wall, and central nervous system are mainly affected by metastasis.[3] At present, chemotherapeutic agents, surgery and radiation are the commonly used treatment strategies in cancer; however they are not fully effective against the high prevalence or low survival rate. Hence, there is a great interest in the development of safe, low-cost anti-cancer agents from natural sources.[4]

Many plants and plant derived agents have been used for cancer treatment since 1950.[4] The phytoconstituents present in the plants are mainly responsible for its cytotoxic activity. The isolation of vincristine and vinblastine from vinca and podophyllotoxins from *Podophyllum hexandrum* are considered as milestones in the development of anti-cancer agents.[6,7] This led to the discovery of other compounds in cancer treatment such as taxanes, camptothecins, and combretastatins.[8,9]

Plant-derived agents act by modulating various signaling pathways in cancer cells. Plant-products are known to modulate multiple signaling pathways simultaneously; hence, they can be very effective in inhibiting uncontrolled cell proliferation of cancer cells, which have multiple survival strategies. Naturally herbs are curative, whereas chemotherapy and cytotoxic drugs are inherently destructive.[10]

*Macrosolen parasiticus* L. Danser (*Loranthaceae*) also known as *Elytranthe parasiticus* or Parasite Honeysuckle is a parasitic shrub, found in the Western Ghats. *M. parasiticus* commonly grows on mango, peepal and jack trees.[11] Phytochemical investigation of the plant revealed the presence of phytosterols, saponins, phenolic compounds
and flavonoids.\textsuperscript{[12]} \textit{M. parasiticus} has been reported to have significant anti-oxidant activity and anti-tumor activity against Ehrlich ascites carcinoma.\textsuperscript{[13,14]} The present study was aimed to investigate the \textit{in vitro} cytotoxic potential of methanolic and aqueous extracts from stem of \textit{M. parasiticus} against MCF-7 breast cancer cells (estrogen receptor [ER] positive) by brine shrimp lethality (BSL) bioassay, MTT assay and sulforhodamine B (SRB) assay.

**MATERIALS AND METHODS**

**Plant material**
The stems of \textit{M. parasiticus} were collected from Manipal, Karnataka, India in the month of September 2009 and were authenticated by Dr. Gopalakrishna Bhat, Taxonomist, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen (PP 565) has been deposited in the museum of Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences (Manipal, India).

**Reagents**
All chemicals and solvents (analytical grade) were obtained from Nice Chemicals, Mumbai. Dulbecco’s minimum essential medium (DMEM) media, 10% fetal bovine serum (FBS) and MTT reagent was purchased from Sigma Aldrich, St. Louis, USA. Tissue culture flasks and 96 well plates were procured from Tarson and Nunc, Sigma Aldrich, St. Louis, USA. Tissue culture flasks and were authenticated by Dr. Gopalakrishna Bhat, Taxonomist, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen (PP 565) has been deposited in the museum of Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences (Manipal, India).

**Plant extract**
The shade dried, coarsely powdered \textit{M. parasiticus} stem (500 g) was extracted with methanol (3 L × 1 L) using soxhlet extractor. For aqueous extract, 500 g of the powdered stem was macerated with water: Chloroform (99:1) for 7 days. The extracts were concentrated and dried \textit{in vacuo} to get the percentage yield of methanolic and aqueous extracts 11.9 and 14.4% w/w, respectively.

**Cell line and culture medium**
Human breast cancer cells (MCF-7) were procured from National Centre for Cell Science Pune, India. MCF-7 cells were grown in 75 cm\textsuperscript{2} tissue culture flasks containing DMEM supplemented with 10% FBS, TPVG solution at 37°C in a CO\textsubscript{2} incubator in an atmosphere of humidified 5% CO\textsubscript{2} and 95% air. The cells were maintained by routine sub culturing in 75 cm\textsuperscript{2} tissue culture flasks.

**Brine shrimp lethality bioassay**
A total of 100 mg of eggs roughly represents 2.5–3.0 1000 of eggs. Hatching chamber was fabricated as per the design used by Meyer \textit{et al}.\textsuperscript{[15]} The chamber was made of glass, with aluminum lid on top. The chamber was divided into two equal parts with the help of a laminated plywood divider having a number of holes of 2 mm size. One of the compartments was illuminated with a lamp (60 W) while the other was darkened. Both the chambers were aerated.

Stock solution was prepared by dissolving 5 mg of sample extracts with 5 mL of dimethyl sulfoxide (DMSO) (1000 μg/mL). From this stock 50 μL, 250 μL and 500 μL were taken and volume was made up to 5 mL (in 7 mL vial capacity) with solution which contain specific volume of brine and yeast suspension to get the final drug concentration of 10 ppm, 50 ppm and 100 ppm. The dilutions were made in triplicates for each dose level. Nauplii was collected using a pipette and transferred 10 such shrimps to each sample vial. The artificial sea water prepared according to the composition given by Dr. Vasudevappa, Research Officer, FRS, Hesaraghatta, Bengaluru. A volume of 5 mL of water and a drop of dry yeast suspension were added to each vial. survivors were counted after 24 h, and LC\textsubscript{50} values were calculated.

**MTT assay**
100 μL of cell suspension of density 1 × 10\textsuperscript{4} cells/well was placed into each well of 96-well plates and incubated for 24 h. Plant extracts were dissolved in DMSO and added (31.25–250 μg/mL) to cultured cells in 96 well plates and incubated for 24 h. Then, the medium was removed and washed with 200 μL phosphate buffered saline (PBS). Added 100 μL of the tetrazolium dye (MTT) solution (1 mg/mL in PBS) to each well of 96 well plates and incubated for 4 h at 37°C. MTT reagent was discarded by inverting the microplates. Formed formazan crystals were dissolved by adding 100 μL of DMSO. The plate was placed in a plate shaker for 5 min. then optical density was read on a microplate reader (BioTek, USA) at 540 nm.\textsuperscript{[16,17]} The experiment was performed in triplicates. Results were expressed as mean ± standard error of the mean (SEM) values.

**Sulforhodamine B assay**
100 μL of cell suspension of density 1 × 10\textsuperscript{4} cells/well was placed into each well of 96-well plates and incubated for 24 h. A range of concentrations (31.25–250 μg/mL) of extracts was made in the culture medium. 100 μL of test sample of each concentration was added to every well except control wells. only 0.1% DMSO was added in the control group. After 48 h, the cells were fixed with ice-cold trichloroacetic acid (100 μL/well, 10% w/v) for 1 h at 4°C. To the washed and dried plates, added 100 μL SRB (0.057% w/v in 1% aqueous acetic acid) solution and
kept at room temperature for 30 min. The unbound SRB solution was removed by washing the plates 5 times with 1% v/v acetic acid. Washed plates were then dried. 200 μL of 10 mM Tris Base (pH 10.5) was added to each well to solubilize the bound SRB. It was then placed in a shaker for 5–10 min. Then, the plates were read in a 96-well plate reader with working wavelength of 570 nm and optical density was noted.[18]

Acridine orange/ethidium bromide staining
Cancer cells were seeded in T-25 flasks and allowed to grow for 24 h. The extracts were added and incubated, then stained with acridine orange (AO), followed by ethidium bromide (EB). The cells were observed under a fluorescent microscope (Polywar, Reinhard Jung).[19]

Results
Brine shrimp lethality bioassay
Both methanolic and aqueous extracts of *M. parasiticus* screened for BSL bioassay were found to be effective. The LD$_{50}$ of methanolic and aqueous extracts was found to be 125 ± 3.04 μg/mL and 82.79 ± 2.67 μg/mL, respectively [Figure 1].

Acridine orange/ethidium bromide staining
Figure 2 shows the morphological examination of MCF-7 cells with dual staining using AO/EB. Cells, which endured apoptosis showed nuclear shrinking and apoptotic bodies. AO gave green color to live nuclei, and dead cells were stained red with EB.

**MTT and sulforhodamine B assay**
The percentage cell growth inhibition by *M. parasiticus* stem extracts at a concentration range of 31.25–250 μg/mL was determined after 48 h. The methanolic and aqueous extracts showed significant activity against MCF-7 breast cancer cells using MTT assay [Figure 3]. Both the extracts demonstrated a dose-dependent inhibition with IC$_{50}$ values of 97.33 ± 3.75 μg/mL and 59.33 ± 3.3 μg/mL, respectively. Results of the SRB assay are presented in Figure 4. As evident from this figure, aqueous and methanolic extracts demonstrated dose-dependent inhibition of cell survival with IC$_{50}$ values of 94.58 ± 3.84 μg/mL and 51.9 ± 1.87 μg/mL respectively. In general, extracts demonstrated cytotoxic activity in a dose depending manner, aqueous extract of *M. parasiticus* was found to be significantly ($P < 0.01$) more effective in both assays compared to methanolic extract. However, methanolic extract demonstrated significant values ($P < 0.01$) against MCF-7 breast cancer cells by SRB assay, compared to MTT assay ($P < 0.05$).

**DISCUSSION**
According to a recent survey by GLOBOCAN project, it was found that nearly 1,55,000 new cases of breast cancer will be diagnosed in 2015 and almost 76000 women in India are likely to die of the disease.[20] As such, we chose to screen *M. parasiticus* extracts for cytotoxicity against MCF-7, an ER positive breast cancer cell line. In general, breast cancer cell lines can be classified as either ER positive or ER negative.[21] The major goal in anti-cancer treatment is the development of molecules with improved therapeutic activity and selectivity. Plant derived agents are used in cancer therapy as a cytotoxic agent. Use of...
nonherbal cytotoxic drug and chemotherapy is often destructive and not curative. Development of cytotoxic drug or formulation using plant products reduces the risk of side effects.

*Macrosolen parasiticus*, known locally as Bandanekke in Kannada is a parasitic plant that belongs to the family *Loranthaceae*. There are numerous plants in this family such as *Viscum album*, *Scurrula ferruginea*, *Helixanthera parasiticus*, *Viscum album coloratum*, *Dendrophthoe Falcata* etc., that are reported to have remarkable anti-cancer potential both *in vitro* and *in vivo*.\(^{[22-27]}\) *M. parasiticus* extract showed anti-oxidant properties against *in vitro* models and also possess anti-cancer properties against Ehrlich ascites carcinoma.\(^{[11-13]}\) The major components of *M. parasiticus* are polyphenols, flavonoids, and saponins, which have anti-oxidant and anti-cancer activity. Plants with anti-oxidant activity show anti-cancer properties by inhibiting the proliferation of multiple human cancer cells.\(^{[14]}\) In animals the dietary polyphenol exhibits anti-carcinogenic activity.\(^{[28]}\)

Moderate level of reactive oxygen species (ROS) are required for cell proliferation, and differentiation, but high levels produce oxidative damage to DNA resulting in mutation; leading to cancer. flavonoids are responsible for preventing the generation of enzymes required for the production of ROS, thus inhibiting over production of ROS and progression of carcinogenesis. Saponins are reported to have antitumor, anti-oxidant and anti-mutagenic activity. Saponins produce cytotoxicity by preventing the growth of cells, which are cancerous, thereby reducing the risk of human cancer. Polyphenols have free radical scavenging activity and protect DNA damage caused by ROS. It also induces apoptosis and inhibits angiogenesis.

The present study suggests that the main compounds such as polyphenols, flavonoids and saponins could be responsible for its cytotoxic activity against MCF-7 breast cancer cell line. Screening of cytotoxicity in cultured cancer cells and in the animal model are necessary to evaluate the anti-cancer potential of plants prior to clinical trials.\(^{[29]}\) Breast cancer cell lines are either ER positive or ER negative. Hence, developing an anti-cancer herbal agent, which is effective to both ER positive and ER negative breast cancer is important to reduce the risk level of breast cancer.

**CONCLUSION**

Significant activity has been observed in both aqueous and methanolic extracts of *M. parasiticus*. Methanolic and aqueous extract of *M. parasiticus* contains phenolic compounds, saponins, and flavonoid.\(^{[11]}\) Cytotoxicity may be due to the presence of chemopreventive constituents in the plant extracts. Further studies on *M. parasiticus* will be carried out to identify the exact compounds responsible for the cytotoxicity and its mechanism of action.

**ACKNOWLEDGMENT**

The authors would like to thank Manipal University and Manipal College of Pharmaceutical Sciences, Manipal, Karnataka, India for providing the necessary facilities to carry out this work.
REFERENCES

1. Kaufman D, Chabner BA. Clinical strategies for cancer treatment: The role of drugs. In: Chabner BA, Longo DL, editors. Cancer Chemotherapy and Biotherapy: Principles and Practice. Philadelphia: Lippincott-Raven; 1996. p. 1-16.

2. Nor-Aini AS, Merrina A, Stanslas J, Sreeramanan S. Cytotoxic potential on breast cancer cells using selected forest species found in Malaysia. Int J Cancer Res 2008;4:103-9.

3. Zia MK, Rmali KA, Mansel RE, Jiang WG. Level of expression of parathyroid hormone related protein and its receptor in human breast cancer and its correlation with the clinical outcome. Int J Cancer Res 2007;3:92-102.

4. Moongkarndi P, Kosem N, Kaslungka S, Luanratana O, Pongpan N, Neungton N. Antiproliferation, antioxidation and induction of apoptosis by Garcinia mangostana (mangosteen) on SKBR3 human breast cancer cell line. J Ethnopharmacol 2004;90:161-6.

5. Kinzler KW, Vogelstein B. Introduction. The Genetic Basis of Human Cancer. 2nd ed. New York: McGraw-Hill Publishers; 2002.

6. Cragg GM, Kingston DG, Newman DJ. Anticancer Agents from Natural Products. USA (FL): Brunner-Routledge Psychology Press, Taylor and Francis Group; 2005.

7. Newman DJ, Cragg GM, Snader KM. Natural products as potential on breast cancer cells using selected forest species found in Malaysia. Int J Cancer Res 2008;4:103-9.

8. Cassady JM, Dourus JD. Anticancer Agents Based on Natural Product Models. 2nd ed. New York: Academic Press; 1980.

9. Pinney KG, Jelina C, Edvardsen K, Chaplin DJ, Petit GR. The Discovery and Development of the Combrestatins. In: Cragg GM, Kingston DG, Newman DJ, editors. Anticancer Agents from Natural Products. Boca Raton (FL): Brunner-Routledge Psychology Press, Taylor and Francis Group; 2005. p. 5-22.

10. Hopking AN. The Use of Herbs and Natural Plant Compounds in the Fight against Cancer. Cancer support association, Information, Wellness and Healing. Available from: http://www.cancersupportwa.org.au/newsletter_article.php?news_id=270. [Last accessed on 2014 Feb 21].

11. Bhat KG. Flora of Udupi. Manipal: Manipal Press Limited; 2003. p. 549.

12. Kodithala S, Yoganandam G, Kiranmai M. Pharmacognostical, phytochemical and anticancer studies of Dendrophthoe falcata plant – A common parasitic medicinal plant. Pharm Sin 2011;2:217-21.

13. Kodithala S, Dashora N, Prabhu K, Lobo R. Antioxidant activities of methanolic and aqueous extract of Macrosolen parasiticus (L.) Danser on ER-positive breast cancer cell line MCF-7. Int J Ayurveda Pharm 2011;2:207-10.

14. Kodithala S, Dashora N, Prabhu K, Lobo R. Evaluation of anticancer activity of Macrosolen parasiticus (L.) Danser on Ehrlich’s ascites carcinoma treated mice. Int J Cancer Res 2011;7:135-43.

15. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med 1982:45:31-4.

16. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.

17. Dua P, Gude RP. Antiproliferative and antiproteolytic activity of pentoxifylline in cultures of B16F10 melanoma cells. Cancer Chemother Pharmacol 2006;58:195-202.

18. Houghton P, Fang R, Techatanawat I, Steventon G, Hylands PJ, Lee CC. The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. Methods 2007;42:377-87.

19. Amarante-Mendes GP, Bossy-Wetzel E, Brunner T, Green DR. Apoptosis assay. In: Spector DL, Goldman RD, Leinwand LA, editors. Cell: A Laboratory Manual. Cold Spring Harbor Laboratory Manual on Cell Biology. New York: Cold Spring Harbor; 1998. p. 15.1-15.4.

20. Ferlay J, Soerjomataram I, Eser S, Mathers C, et al. GLOBOCAN 2012. v1.0, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://www.globocan.iarc.fr. [Last accessed on 2014 Dec 11].

21. Swami S, Raghavachari N, Muller UR, Bao YP, Feldman D. Vitamin D growth inhibition of breast cancer cells: Gene expression patterns assessed by cDNA microarray. Breast Cancer Res Treat 2003;80:49-62.

22. Graham JG, Quinn ML, Fabricant DS, Farnsworth NR. Plants used against cancer – An extension of the work of Jonathan Hartwell. J Ethnopharmacol 2000;73:347-77.

23. Kohhizic-Le Dévéhat F, Tomasi S, Fontanel D, Boustie J. Flavonoids from Scruella ferruginea Danser (Loranthaceae). Z Naturforsch C 2002;57:1092-5.

24. Ldrdpapamongkol K, Mahidol C, Thongnest S, Prawat H, Ruchirawat S, Srisomsap C, et al. Anti-metastatic effects of aqueous extract of Helixanthera parasitica. J Ethnopharmacol 2003;86:253-6.

25. Yoon TJ, Yoo YC, Kang TB, Baek YJ, Huh CS, Song SK, et al. Prophylactic effect of Korean mistletoe (Viscum album coloratum) extract on tumor metastasis is mediated by enhancement of NK cell activity. Int J Immunopharmacol 1998;20:163-72.

26. Lobo R, Dashora N, Sodde V, Bhagat J, Prabhu KS. Antitumor activity of Dendrophthoe falcata against Ehrlich ascites carcinoma in Swiss albino mice. Pharm Crops 2011;2:1-7.

27. Kodithala S, Yoganandam G, Kiranmai M. Pharmacognostical, phytochemical and anticancer studies of Dendrophthoe falcata (L.f.) ettingsh. (Loranthaceae) growing on the host plant Azadirachta indica (Meliaceae). Int J Pharm Biol Sci 2013;4:1010-8.

28. Ju EM, Lee SE, Hwang HJ, Kim JH. Antioxidant and anticancer activity of extract from Betula platyphylla var. Japonica. Life Sci 2004;74:1013-26.

29. Singh AV, Xiao D, Lew KL, Dhir R, Singh SV. Sulforaphane induces caspase-mediated apoptosis in cultured PC-3 human prostate cancer cells and retards growth of PC-3 xenografts in vivo. Carcinogenesis 2004;25:83-90.

Cite this article as: Sodde VK, Lobo R, Kumar N, Maheshwari R, Shreedhara CS. Cytotoxic activity of Macrosolen parasiticus (L.) Danser on the growth of breast cancer cell line (MCF-7). Phcog Mag 2015;11:156-60.

Source of Support: Nil, Conflict of Interest: None declared.