

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

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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.^[1] 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.^[5] 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

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and flavonoids.^[12] *M. parasiticus* has been reported to have significant anti-oxidant activity and anti-tumor activity against Ehrlich ascites carcinoma.^[13,14] The present study was aimed to investigate the *in vitro* cytotoxic potential of methanolic and aqueous extracts from stem of *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 *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, USA). Trypsin phosphate versene glucose (TPVG) solution (Himedia, Mumbai) were used in the present study.

Plant extract

The shade dried, coarsely powdered *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 *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 cm² tissue culture flasks containing DMEM supplemented with 10% FBS, TPVG solution at 37°C in CO₂ incubator in an atmosphere of humidified 5% CO₂ and 95% air. The cells were maintained by routine sub culturing in 75 cm² 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 *et al.*^[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 LC₅₀ values were calculated.

MTT assay

100 µl of cell suspension of density 1 × 10⁴ 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.^[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 × 10⁴ 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 μ L 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]

Statistical analysis

All the experiments were performed in triplicates. Data were represented as mean \pm SEM values. The IC_{50} or LD_{50} values were obtained by nonlinear regression using Microsoft Excel spread sheet application. Statistical analysis of the data were performed by one-way ANOVA (GraphPad Prism 5.02, GraphPad Software, Inc., California) followed by Tukey's test.

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 $125 \pm 3.04 \mu\text{g/mL}$ and $82.79 \pm 2.67 \mu\text{g/mL}$, respectively [Figure 1].

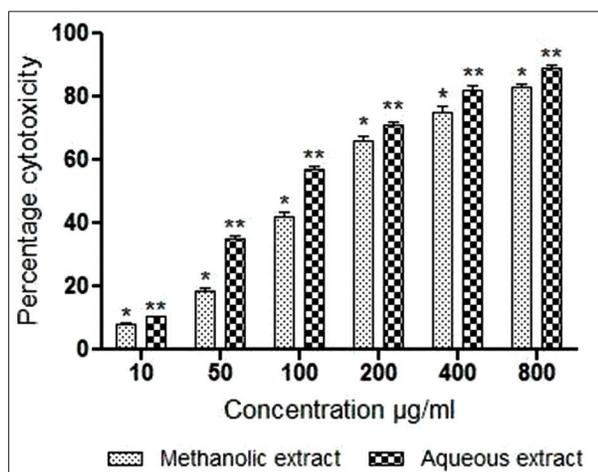


Figure 1: Effect of various extracts of stem of *Macrosolen parasiticus* on brine shrimp lethality. Results were expressed as mean values \pm standard deviation of independent experiments performed in triplicate. The values were significant at $P < 0.05$ and $P < 0.01$ (indicated by single asterisk [*] and a double asterisk [**] respectively) compared to control using one-way ANOVA followed by Tukey's test

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 $\mu\text{g/mL}$ 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 $97.33 \pm 3.75 \mu\text{g/mL}$ and $59.33 \pm 3.3 \mu\text{g/mL}$, 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 $94.58 \pm 3.84 \mu\text{g/mL}$ and $51.9 \pm 1.87 \mu\text{g/mL}$ 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

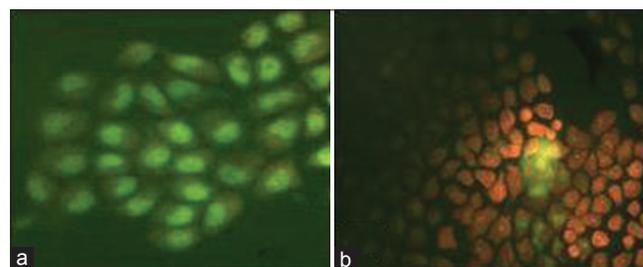


Figure 2: Fluorescence microscopy of MCF-7 stained with acridine orange/ethidium bromide after treatment with *Macrosolen parasiticus* extract. (a) Normal cell; (b) Treatment with extracts led to nuclear shrinking and formation of apoptotic bodies

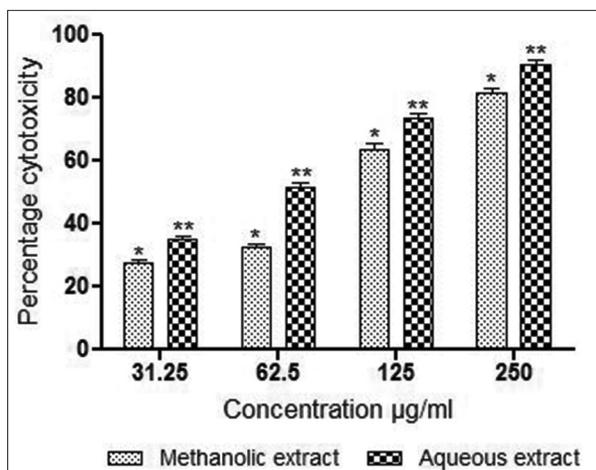


Figure 3: *In vitro* cytotoxic activity of methanolic and aqueous extracts of stem of *M. parasiticus* in MCF-7 breast cancer cells (estrogen receptor positive) by MTT assay at 48 h of exposure. Results were expressed as mean values \pm standard deviation of independent experiments performed in triplicate. The values were significant at $P < 0.05$ and $P < 0.01$ (indicated by single asterisk [*] and double asterisk [**] respectively) compared to control using one-way ANOVA followed by Tukey's test

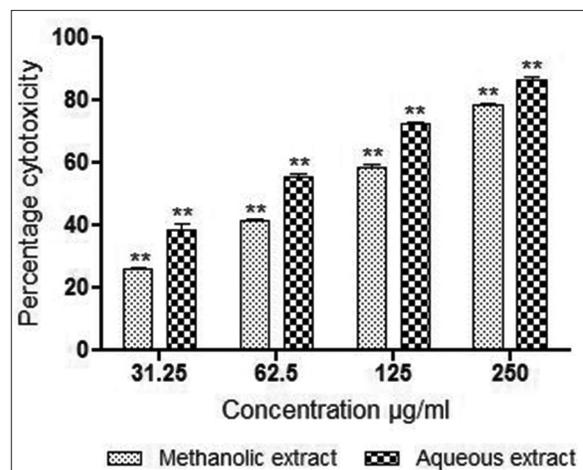


Figure 4: *In vitro* cytotoxic activity of methanolic and aqueous extracts of stem of *Macrosolen parasiticus* in MCF-7 breast cancer cells (estrogen receptor positive) by sulforhodamine B assay at 48 h of exposure. Results were expressed as mean values \pm standard deviation of independent experiments performed in triplicate. The values were significant at $P < 0.05$ and $P < 0.01$ (indicated by single asterisk [*] and double asterisk [**] respectively) compared to control using one-way ANOVA followed by Tukey's test

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.

M. 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.

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