

ANTIPROLIFERATIVE EFFECT OF MERREMIA EMARGINATA (burm.F.) LEAF EXTRACT ON SAOS-2 CELL LINE

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Abstract

Introduction: One of the most prevalent malignant tumors is osteosarcoma. The use of herbal medicines that can suppress or kill cancer cells through their antioxidant properties is growing in popularity nowadays as a result of the numerous negative effects of cancer medications. Different parts of *M.emarginata* have been mentioned to be therapeutically used as diuretic and neuralgia. The main aim of this study is to find out an antiproliferative activity of *M.emarginata* leaf extract on SAOS 2 cell line

Materials and Methods: The *Merremia emarginata*(burm.F.) leaf were soxhlet extracted. Cultured Saos Cells were stimulated with various concentrations of *M.emarginata*(burm.F.) leaf extracts. To dissolve the formazan formed from MTT, the cells were resuspended in dimethyl sulfoxide (DMSO). The inhibitory rate of cell growth was calculated. mRNA expression levels were examined using real-time PCR.

Results: As the concentration of leaf extract increases from 50µg/ml to 200µg/ml the cell viability is significantly decreased. Bcl2 and Bcl-xl expression were significantly negatively correlated with higher concentration of Saos -2 cells treated with *M.emarginata*.

Conclusion: The study revealed that *M.emarginata* has a significant role in controlling bone cancer cell proliferation by upregulating caspase 3 mRNA and down regulating the gene expression of Bcl 2 and Bcl xl.

Keywords: Anti-proliferative, anti-cancer, apoptosis, bone cancer, Leaf extract, *Merremiaemarginata*.

INTRODUCTION

Globally, cancer is a diverse genetic condition that causes significant health issues. Cancer is the second leading cause of death in India. A relatively high mesenchymal tumor called osteosarcoma is defined by spindle cells that deposit an underdeveloped osteoid matrix [1]. Aside from leukemia and lymphoma, osteosarcoma is currently the most common primary bone cancer in both children and adolescents [2]. Because osteosarcomas have a high likelihood of metastasizing, even though they only constitute nearly 6 % of all juvenile tumors, they are among the most common causes of cancer-related death [3]. The molecular composition of the osteosarcoma extracellular matrix is not well characterized. Saos-2 cell has the most mature osteoblastic labeling profile. Only patients with low-grade tumors typically benefit from surgical resection [4]. Other therapeutic approaches, like chemo or radiation therapy, must be used for patients with high-grade malignancies [5]. In order to treat or prevent cancer, antioxidant chemicals obtained from herbal extracts are preferred over prescribed drugs and therapy because they weaken the immune system and have several adverse effects [6,7]. Numerous antioxidant substances included in herbal medicine combat free radicals and inhibit lipid peroxidation. Secondary metabolites found in plants include phenol and flavonoids. These molecules have antioxidant characteristics and a significant ability to remove free radicals, preventing diseases like cancer [8].

Modern pharmaceutical substances were developed on the utilization of plant extracts and pure, isolated molecules from natural sources. Researchers from all over the world are actively involved in the creation of plant-based anticancer medicines. They screened and analyzed a variety of plants and were able to identify thousands or more of them as having substantial anticancer

qualities. *Merremiaemarginata* (Burm. f.) is a member of the Convolvulaceae family of plants. *Ipomoea reniformis* chois is another name for the *Merremiaemarginata* plant. It is primarily found in Chennai and a few locations in Andhra Pradesh in India. It seems to have a variety of significant therapeutic effects. *Merremiaemarginata* has been promoted as beneficial in the Indigenous medical system for treating fever caused by liver enlargement, cough, headache, neuralgia, rheumatism, diuretic, inflammation, nose problems, and renal ailments. The root contains a diuretic and a laxative and is used to treat eye and gum disease. The powder from the leaves is used as a snuff during epilepsy spasms. Varieties of compounds are derived from *Merremiaemarginata* such as flavonoids, tannins and phenolic compounds. *M.emarginata* extract is a significant source of natural antioxidants and exhibits free radical scavenging activities. The investigations on toxicity indicated that most *Merremia* species are safe for human use but not prolonged chronic administration [9]. Our team has extensive knowledge and research experience that has translate into high quality of publications [10–23]

The study was designed to determine an anti-proliferative effect on the Saos2- cell line using *Merremiaemarginata* leaf extract.

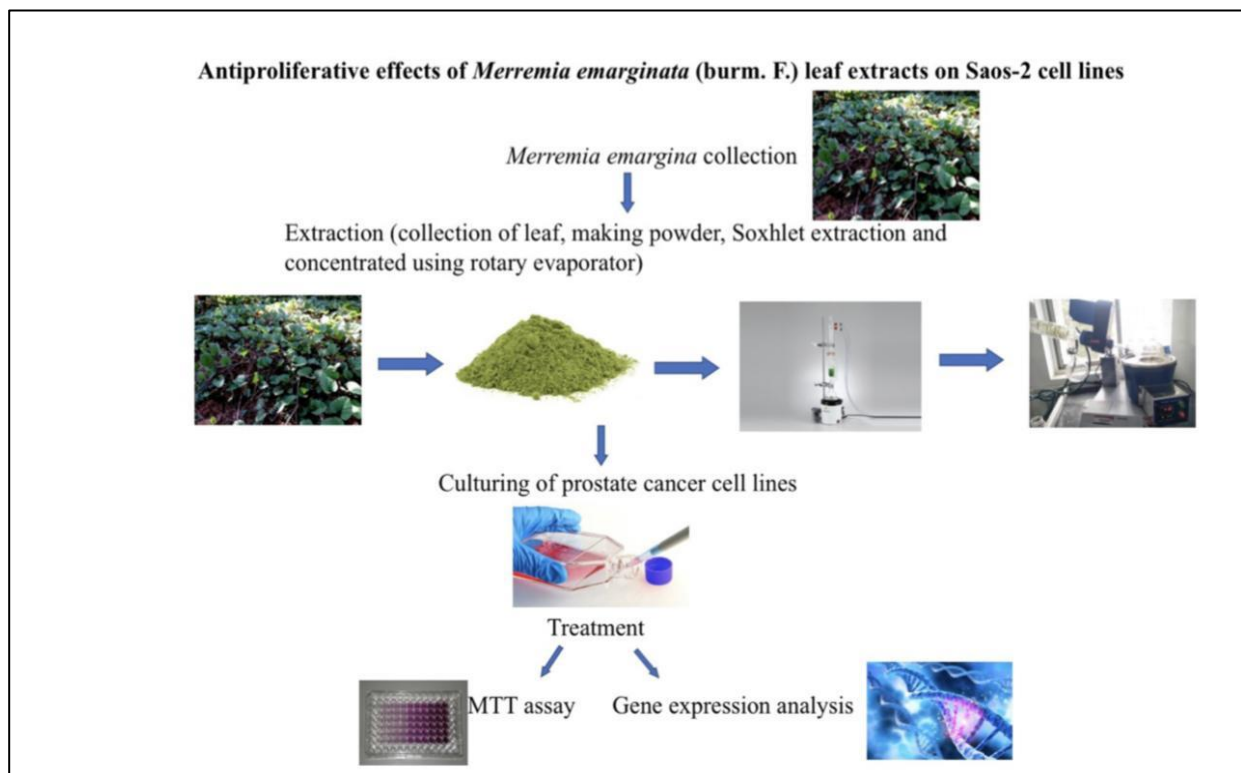
MATERIALS AND METHODS:

Chemicals:

Trypsin-EDTA, fetal bovine serum (FBS), antibiotics-antimycotics, Dulbecco's modified Eagle's medium (DMEM) and phosphate buffered saline(PBS) were purchased from Gibco, Canada. JC-1 (5,5,6,6- tetrachloro- 1,1,3,3- tetraethyl benzimidazole carbocyanine iodide) and real-time PCR kit (MESA Green) were purchased from invitrogen, USA. All the chemicals used were extra pure of analytic grade.

Extract preparation:

The *Merremia emarginata*(burm.F.) leaf was soxhlet extracted with 70% ethanol. The extract was then filtered with Whatman no.1 filter paper and the solvent evaporated at reduced pressure by using a Rotary evaporator apparatus to get a viscous mass, which was then stored at 4° until used.

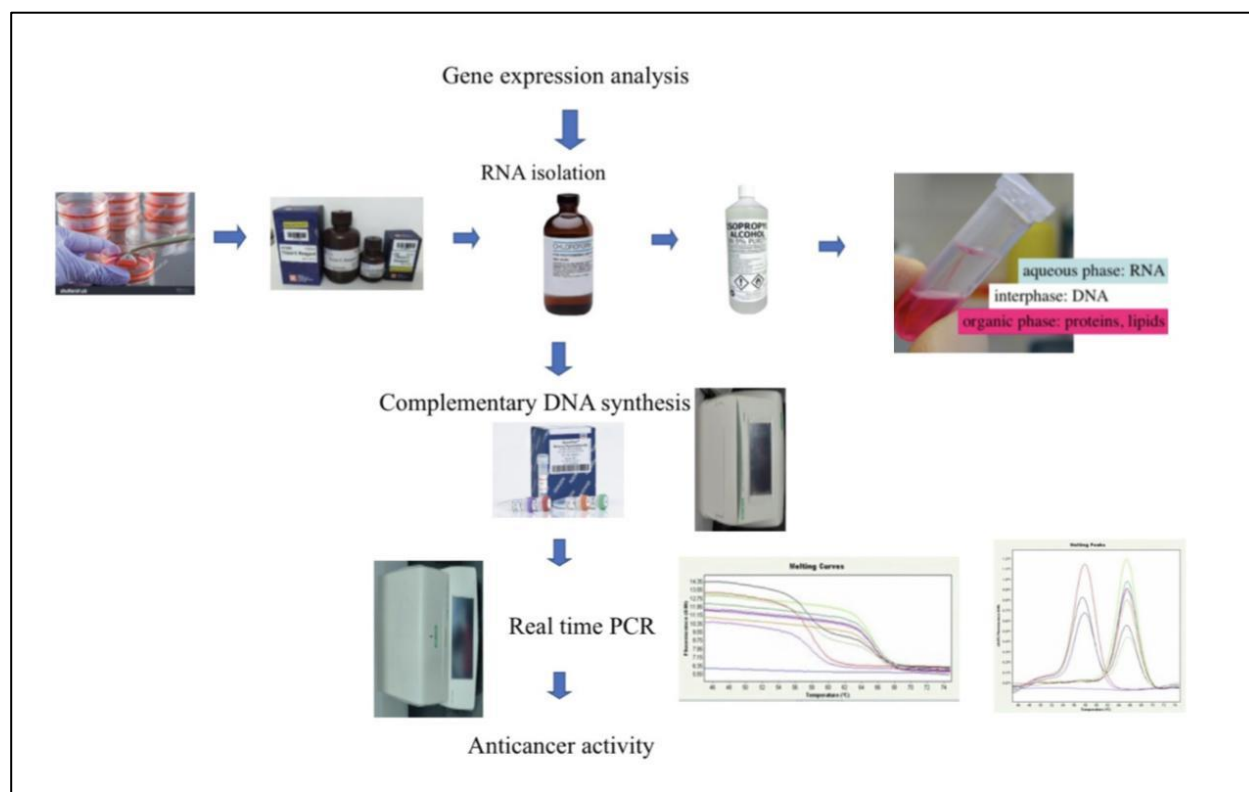


Saos2 cells were seeded at the density of 5×10^5 cells/well in 96-well plates and allowed to attach to the well overnight. After incubation, cultured cells were stimulated with various concentration of *M.emarginata*(burm.F.) leaf extracts in triplicate and incubated at 37° in a 5% humidified CO₂ incubator for 24hours. Subsequently, 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well, and incubation was continued for a further 4h at 37°C . To dissolve the formazan formed from MTT, the cells were suspended in 200 μl dimethyl sulfoxide (DMSO), and the optical density (OD) of the solution was determined using a spectrometer at a wavelength of 570 nm. The experiments were repeated 3 times, independently. The mean optical density (OD) \pm SD for each group of replicates was calculated. The entire procedure was repeated 3 times. The inhibitory rate of cell growth was calculated using the equation:

$$\% \text{ Growth inhibition} = (1 - \text{OD extract treated}) / \text{OD negative control} \times 100.$$

Gene expression analysis by Real Time PCR:

mRNA expression levels were examined using real-time PCR. The total RNA was isolated by using Tri Reagent (Sigma). Total RNA (2 μg) from each sample was reverse transcribed using a commercial SuperscriptIII first strand cDNAsynthesis kit (Invitrogen, USA) according to the manufacturer's protocol. The data were analyzed by comparative CT method and the fold change is calculated by $2^{-\text{CT}}$ method described by Schmittgen and Livak (2008) using CFX Manager Version 2.1 (Bio Rad, USA). Data were expressed as the means \pm SD of 3 individual experiments performed in triplicate. Statistical analysis was performed using the one-way ANOVA and $p < 0.05$ was considered to indicate a statistically significant result.



RESULTS:

As the concentration of leaf extract increases from $50\mu\text{g/ml}$ to $200\mu\text{g/ml}$ the cell viability is significantly decreased. Bcl2 and Bcl-x1 expression were significantly negatively correlated with higher concentration of Saos -2 cells treated with *M.emarginata*. Based on the results showed that Caspase 3 was positively correlated with *M.emarginata*.

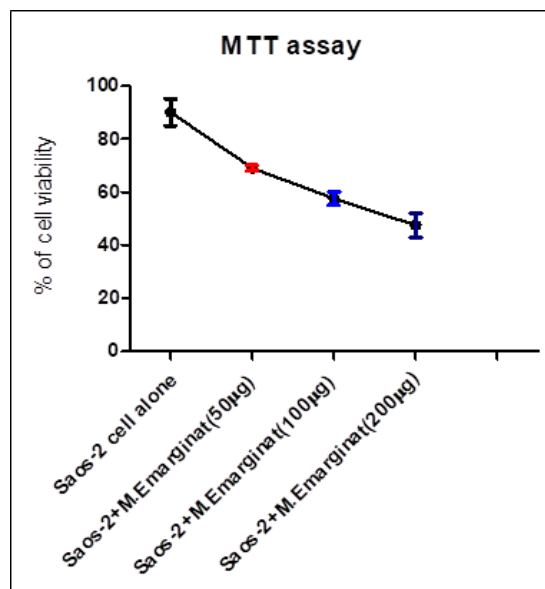


Figure 1: MTT assay

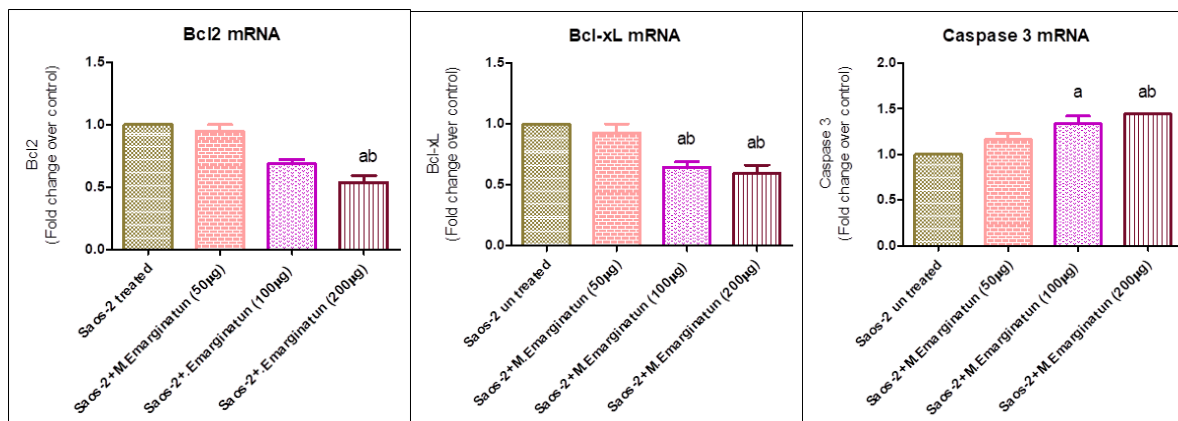


Figure 2: Bcl2, Bcl-xl mRNA expression

DISCUSSION:

The statistical analysis of the data in Figures 1 revealed that *M.emarginata* extracts seemed to have a time- and dose-dependent cytotoxic effect on Saos-2 cells. At a concentration of 200 µg/ml of *M.emarginata* leaf extract, with a percentage of 50% cell growth inhibition, the largest amount of cancer cell growth was inhibited. As the concentration of leaf extract increases from 50µg/ml to 200µg/ml the cell viability is significantly decreased. Secondary plant metabolites like phenols and flavonoids, which are antioxidants, have a significant potential to treat and prevent cancer [24]. *Merremiaemarginata* was discovered to exhibit cytotoxic activity in its various sections, however only the ethyl acetate fraction with an IC₅₀ value under 200 µg/ml was found to be active. When the other fractions were evaluated, the IC₅₀ value greater than 200 µg/ml was deemed inactive [25].

Bcl2 mRNA showed significant reduction at 200µg/ml of treated *M.emarginata*. Bcl-xl mRNA showed a significant reduction in the concentration of 100µg/ml and 200µg/ml. Caspases during apoptosis and recombinant caspase-3 in vitro both cleave the apoptosis-inhibiting Bcl-2 protein at Asp-34. Bcl-xl was localized mainly in the cytoplasm and mitochondria of both normal and cancer cells. Bcl-xl inhibits the activation, preventing a loss of mitochondrial outer membrane integrity and release of cytochrome c into cytoplasm. It is found in tissues containing long lived postmitotic cells [26]. The *M.emarginata*-treated Saos-

2 cell showed a significant reduction in the proliferation of bone cancer cells by regulating the expression of caspase 3 mRNA (Figure 2). A large number of medicinal plants and their purified constituents have been shown beneficial therapeutic potentials. One of the essential components of natural substances' anticancer effects is the modification of apoptotic signaling pathways [27]. Caspase 3 is a lysosomal enzyme involved in the detection of apoptotic pathways and is more specific in the detection of apoptotic cells [28]. As a result, Bcl-2 cleavage by caspase-3 appears to induce more caspase activation as a component of a vicious feedback loop for killing the cell.

CONCLUSION:

According to our research, *M. emarginata* destroys bone cancer cells by inducing apoptosis via the Bax/Bcl2 and caspase 3 pathways. The study revealed that *M.emarginata* has a significant role in controlling bone cancer cell proliferation by upregulating caspase 3 mRNA and down regulating the gene expression of Bcl 2 and Bcl-xl.

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Author Contributions:

Janani K S: Literature search, Data collection analysis, Manuscript drafting

Dr.R. Gayatri Devi :Data Verification, Manuscript draft

Dr.J.Selvaraj: Data collection analysis, Data Verification, Manuscript draft

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