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Antioxidant and Antiproliferative properties of *Aegle marmelos* leaves extract

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ABSTRACT

Aeglemarmelos Linn (family *Rutaceae*.) is indigenous to India and is used in folk medicines. However, *Aeglemarmelos* has not been much explored for its antioxidant and antiproliferative activities. Hence, in the present study an attempt has been made to evaluate the *in vitro* antioxidant activity, antiproliferative activity against breast cancer and nutritional value of *Aeglemarmelos* leaves extract. The free radical scavenging activity of the leaves extract was determined against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, 2,2'-azino-bis(3- ethylbenzothiazoline-6-sulphonic acid) ABTS radical, Super Oxide and Nitric oxide scavenging assays. At a concentration of 1000µg/ml, the leaves extract significantly scavenged 85.50 % of DPPH radicals and 81.50 % ABTS radicals. The leaves extract exhibited a maximum of 86.1% Superoxide scavenging activity and 82.6% nitric oxide and 73% hydroxyl radical scavenging activity. The ethanolic extract of *Aeglemarmelos* (20-200µg/ml) inhibited the cell proliferation by more than 85% at its maximum concentration with an IC_{50} of 54.19 µg/ml. The present study reveals the presence of active bioingredients in the leaves extract. In addition to it, *Aegle marmelos exhibited* effective antioxidant and antiproliferative activity against breast cancer. Hence, *Aegle marmelos can* be used in the treatment of breast cancer.

KEY WORDS: *Aegle marmelos*; Antioxidant; Antiproliferative activity.

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INTRODUCTION

Breast cancer is a serious and significant public health crisis which is predominant in both developed and developing countries[1,2]. It is the most frequent type of nonskin malignancy among women worldwide. In general, conventional treatment options for cancer (i.e., surgery, radiotherapy, chemotherapy, biological therapy, and hormone therapy) are not completely effective. Recurrence and other pathologic situations of breast cancer are still becoming an issue in patients due to side effects, toxicity of drugs in normal cells, and aggressive behaviour of the tumors. Hence, recognition of medicinal plants as effective and inexpensive sources of synthetic novel chemotherapeutic compounds is increasing at an alarming rate in the last decades[3].

McLay *et al*., has reported that 38% of treated breast cancer patients (in a total of 360 questionnaires) use herbal formulations which interact with adjuvant endocrine therapies[4]. In the series of medicinal plants, *Aeglemarmelos* is one such important medicinal plant well known for its folkloric use.

Aegle marmelos (L.) Correa, commonly known as bael (or bel), belonging to the family Rutaceae, is a moderate-sized, slender and aromatic tree. It is indigenous to India and is abundantly found in the Himalayan tract, Bengal, Central and South India. It has several pharmacological activities such as anti-inflammatory, antipyretic, analgesic, antioxidant[5], and ant diabetic property[6]. In view of medicinal importance of *A.marmelos*, in the present study an attempt has been made to evaluate the antioxidant and antproliferative effects of *Aegle marmelos leaves* extract.

MATERIAL AND METHODS

Plant material

The leaves of *A. marmelos*was collected from the surroundings of Villivakkam, Chennai. The plant was identified and authenticated by a taxonomist at the Centre for Advanced studies in Botany, University of Madras.

Preparation of extract

The leaves of *A. marmelos* were dried at room temperature and powdered in an electrical grinder, which was then stored in an airtight container at 5° C until further use. Almost all the chlorophyll and lipid is deposited on the side of the flask and was removed carefully. The extraction was done with ethanol. The dried powder was subjected to Soxhlet using ethanol. The ethanolic extract was filtered, dried and weighed. The extract obtained was evaporated in rotary evaporator to get a powdery mass. The extract was dried under reduced pressure using rotary evaporator to get the crude. It was stored below 4 ºC for further use.

Invitro antioxidant assays

Free radical scavenging assay

The free radical scavenging capacity of the ethanolic extract of *A. marmelos* was determined using DPPH, ABTS, NO, Superoxide radical and Hydroxyl radical scavenging assay[7,8,9,10,11].

Antiproliferative effects of A.marmelos leaves extract

Cell culture

MCF7 (Breast cancer cells) was procured from NCCS, Pune, India. The cells were seeded in a 25-cm^2 flask in DMEM medium with 10% FBS, 1% antibiotic and antimycotic solution at 37°C in 5% CO_2. The cell culture media was changed on alternative days. Upon reaching confluence, the cells were passaged (passage number: 25-29) and were used for cytotoxicity evaluation.

Assessment of cytotoxicity (MTT assay)

The cytotoxicity of *A.marmelos* leaves extract was assessed using MTT assay with some modifications[12]. The cell count was assessed using Trypan blue exclusion test and around 15000 cells were seeded in a flat bottomed 96 well plates and was supplemented with 100µl of DMEM with 10% FBS, 1% antibiotic and antimycotic solution at 37°C in 5% CO_2. The cells were treated with ethanolic extract (concentration range of 25, 50, 75, 100, 125, 150, 200, 300, 400 and 500µg/ml of *A. marmelos* leaves extract. After 24 hours of incubation, 10µL of MTT was added to the plate and incubated for 4 hours at 37°C in 5% CO_2. Then the formazan crystals were dissolved using DMSO and the violet colour was observed using a microplate reader (BIORAD) at 570nm. The absorbance of untreated cells was considered as 100% viable, cell cytotoxicity was calculated based on the formula,% Cytotoxicity = 1-[mean absorbance of treated cells/mean absorbance of negative control]×100

Statistical analysis

All experiments were performed in three different sets with each set duplicated. The data are expressed as the mean ± standard deviation.

RESULTS AND DISCUSSION

In vitro antioxidant activity of A.marmelos

Free radicals which are involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathogens such as cancer and cardiovascular diseases among others[13]. Antioxidants derived from plants provide protection to cell by scavenging free oxygen radical through offsetting ROS. This has been made possible due to the presence of certain bioactive substances, such as phenolic compounds, flavonoids, and essential oils, rendering plants with

antioxidant activity[14].The DPPH radical scavenging has been widely used to evaluate the free radical scavenging ability of various natural products and has been accepted as a model compound for free radical originating in lipids[15].

The DPPH and ABTS radicals scavenging activity of *A.marmelos* is depicted in Figure 1 and 2 respectively. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was quantified in terms of percentage inhibition of a pre-formed free radical by antioxidants. Likewise, ABTS radical activity was quantified in terms of percentage inhibition of the ABTS radical cation by the antioxidant.

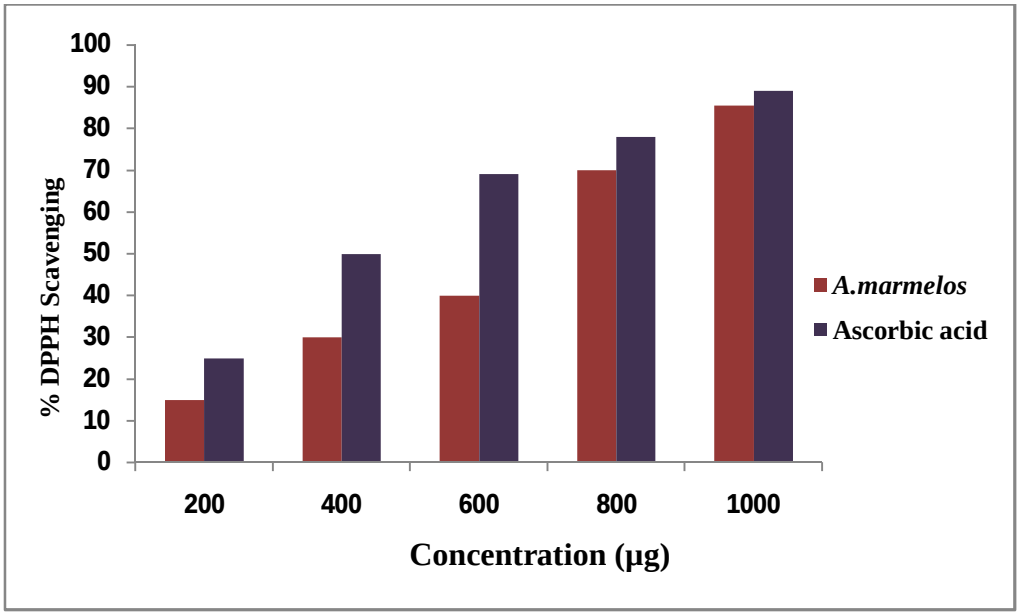

Figure 1: DPPH scavenging potential of *A.marmelos* leaves extract

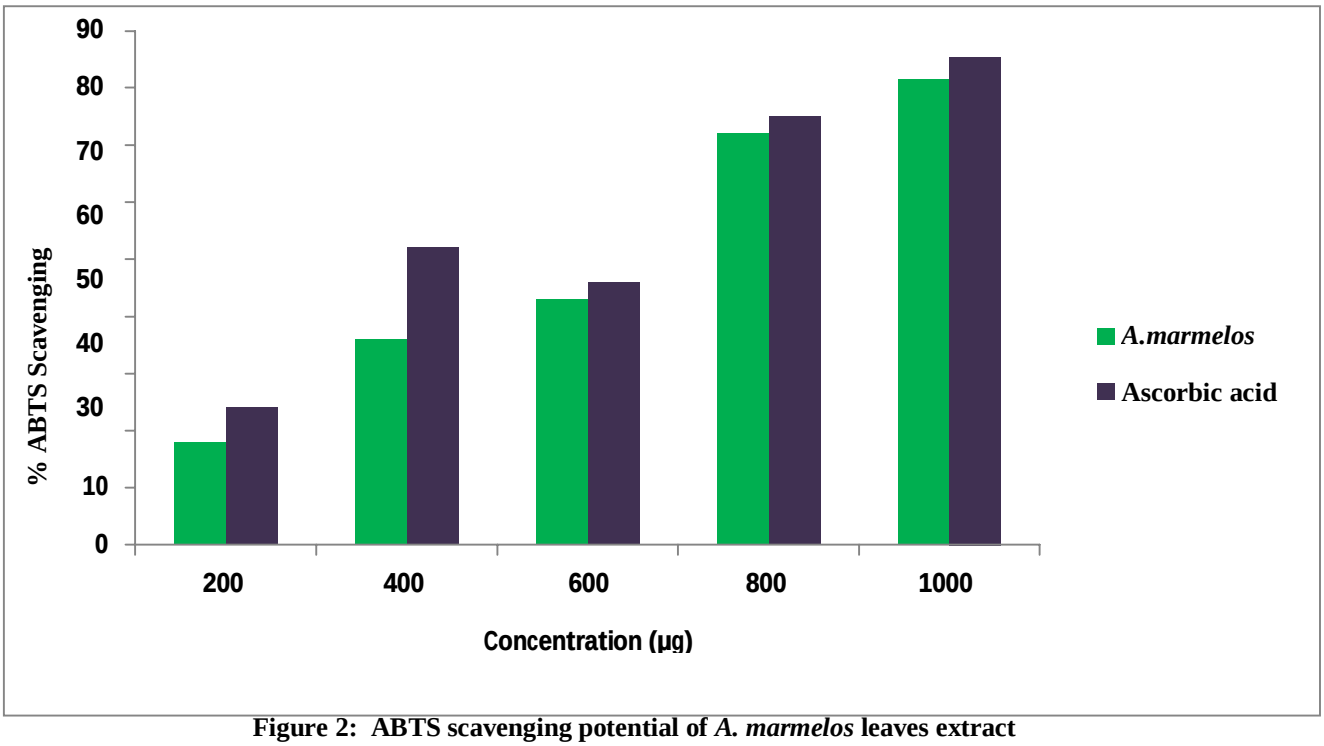

Figure 2: ABTS scavenging potential of *A. marmelos* leaves extract

Among the several *in vitro* antioxidant assays, DPPH and ABTS radical assays have been widely used as more convenient and reliable methods in determining the free radical scavenging efficacy of the lead molecules[16,17]. *A.marmelos* showed 85.5% inhibition at a concentration of 1000 µg in DPPH assay and 81.5% inhibition at a concentration of 1000 µg in ABTS radical assay reflecting its significant radical scavenging capacity.

The *in vitro* superoxide scavenging activity of *A.marmelos* is graphically represented as figure 3. *A.marmelos* exhibited a maximum of 86.1% superoxide scavenging activity at a concentration of 1000µg.

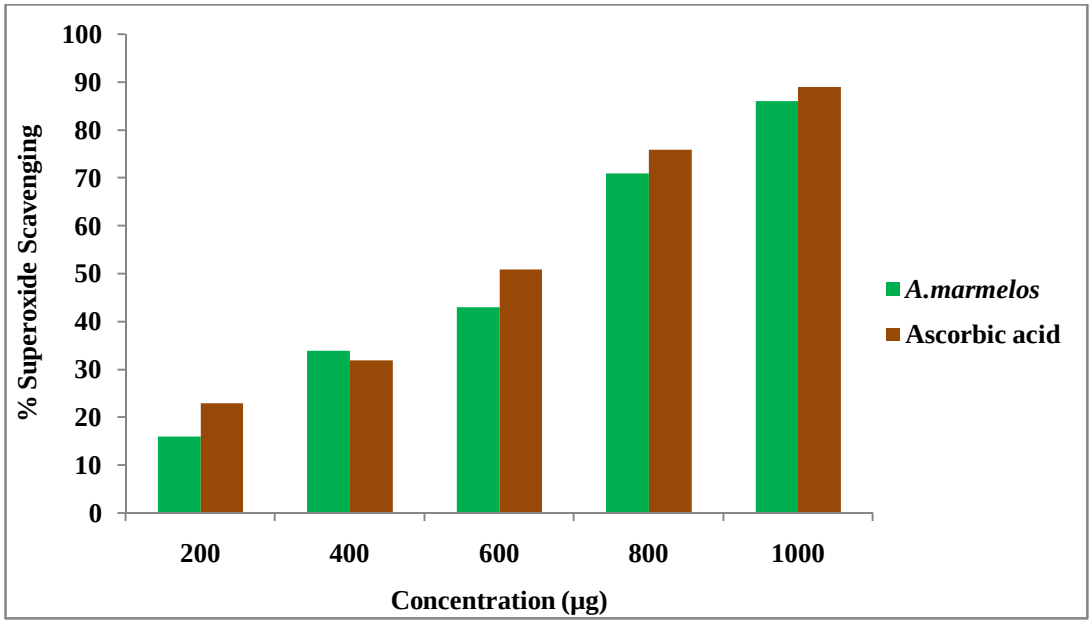

Figure 3: Superoxide scavenging potential of *A. marmelos* leaves extract

The primary free radical in most biological systems are superoxide (O2•−). Superoxide anion radicals (O2˙−) are formed from molecular oxygen by the addition of an electron. The results of the present study established that *A.marmelos* scavenged O2•− and NO significantly and in a concentration-dependent manner.

Nitric oxide is synthesized from L-arginine by the enzyme nitric oxide synthase (NOS). It plays an important role in N-methyl-D-aspartate (NMDA) receptor activation and the induction of significant oxidative stress. NO induced oxidative stress causes lipid peroxidation and neuronal cell death by DNA damage[18,19]. *A. marmelos* plays a crucial role in ameliorating the oxidative stress by reducing the excessive generation of potent free radicals. The data obtained evidenced that *A.marmelos* possesses NO scavenging effect or the inhibition of NO generation. *A.marmelos* at a concentration of 1000µg/ml quenched 82.6% NO radical (Figure 4).

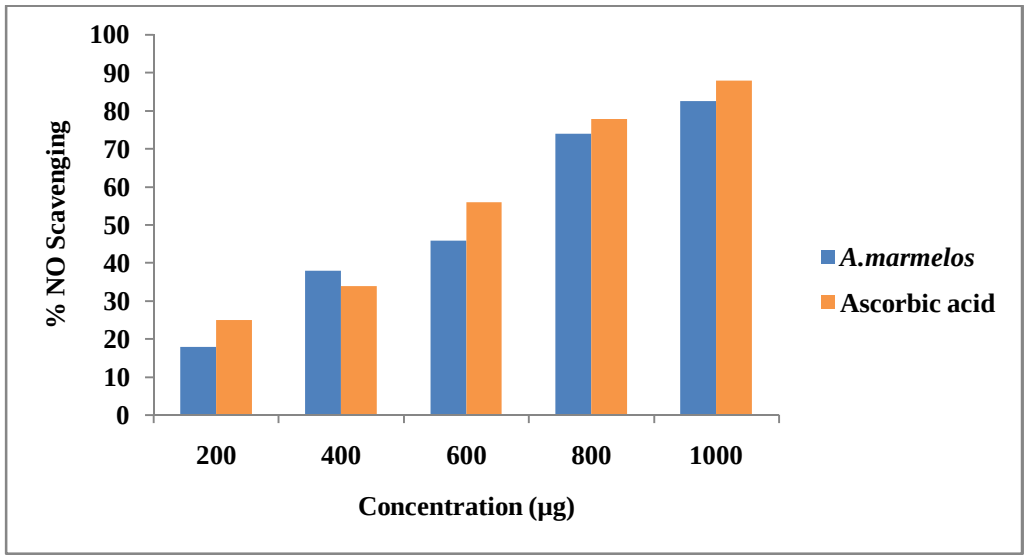

Figure 4: NO scavenging potential of *A. marmelos* leaves extract

Hydroxyl radical scavenging activity of *A.marmelos* is graphically represented in figure 5. Hydroxyl radicals are one of the potent reactive oxygen species in the biological system that is capable of reacting with polyunsaturated fatty acids present in the cell membrane phospholipids and causes extensive damage to the cell[20].

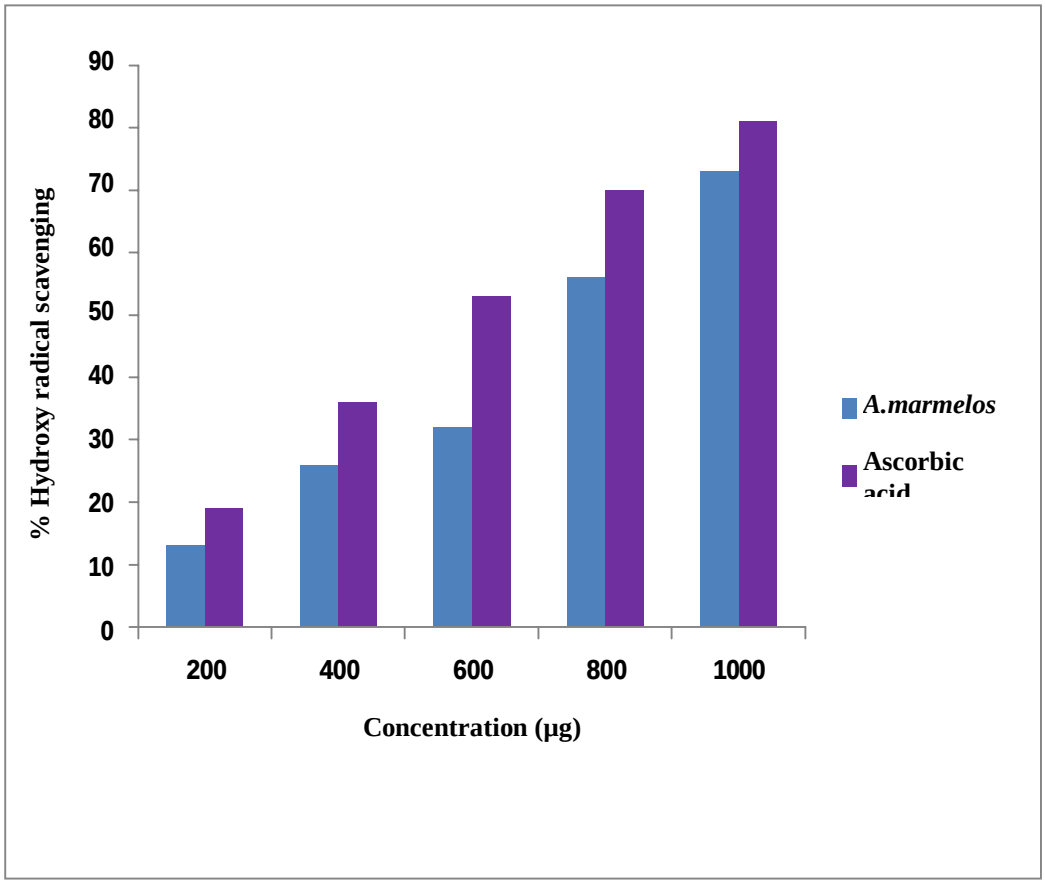

Figure 5: Hydroxyl radical scavenging potential of *A.marmelos* leaves extract

The maximum activity observed in *A. marmelos* could be due to the flavonoids present in them. *A. marmelos* has been reported to contain aegelinine, cineole which possess antioxidative and free radical scavenging activity. It has been reported that the alcoholic leaf extract of *A.marmelos* showed better activity with NO radical and inhibited the generation of anions[21]. Our results are in accordance with the results of Pawaskar and Sasangan[22].

A. marmelos bear potent antioxidant activity. The antioxidants act as defense mechanism that protects against oxidative damage and includes compounds to remove or repair damaged molecules and sufficient intake of antioxidants is supposed to protect against diseases. The phytochemical antioxidants have potent potential to neutralize free radicals or oxidants responsible for the oxidative stress induced cell damage. The present study thus scientifically validates and strengthens the candidature of *A. marmelos* in the preparation of medicinal aids to combat the myriad diseases arising due to oxidative stress.

Antiproliferative effects of Aegle marmelos leaves

MCF-7 breast cancer cell lines are known to be the hormone responsive cell lines which are widely used as one of the models for breast cancer studies. Antiproliferative effects of *A.marmelos* leaves extract was assessed by MTT assay. The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. It depends both on the number of viable cells and on the mitochondrial activity of cells. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay is based on the assumption that dead cells or their products do not reduce tetrazolium. Tetrazolium salts are reduced only by metabolically active cells. Thus MTT can be reduced to a blue coloured formazan by mitochondrial enzyme succinate dehydrogenase. The amount of formazan produced is directly proportional to the number of active cells.

The ethanolic extract of *A. marmelos* leaves showed significant cytotoxicity effect against MCF-7 breast cancer cells in 24 hours. The ethanolic extract (20-200µg/ml) inhibited the cell proliferation by more than 85% at its maximum concentration with an IC_{50} of 54.19 µg/ml(Figure 6). The results suggested that ethanolic extract of *A. marmelos* leaves inhibited the growth of MCF-7 breast cancer cells in a very effective way.

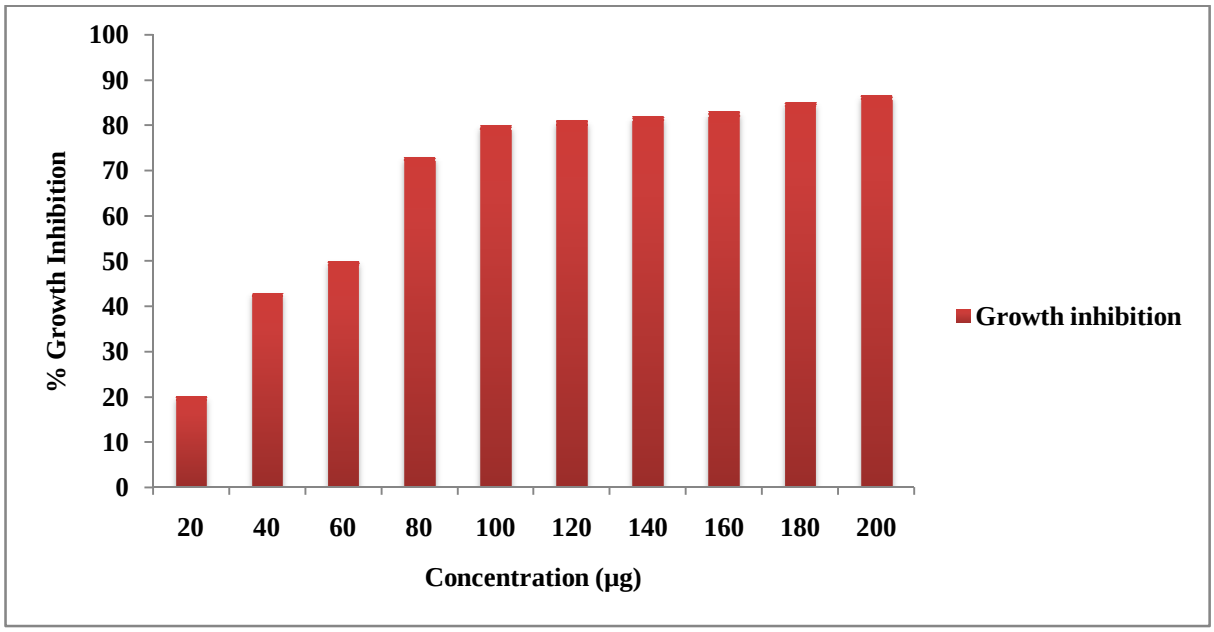

Figure 6: Toxicity effects of the ethanolic extract of *A. marmelos* leaves extract against cancer cell line (MCF-7) after 24 hours of incubation

It has been reported that phytochemicals from *A.marmelos* such as lupeol, eugenol, citral, cineole and D-limonene possess antineoplastic effects. Marmelin also have shown inhibition on AKT and extracellular signal regulated kinase phosphorylation both in cells in culture and in tumor xenografts. AKT plays a key role in tumor cell survival, proliferation, and invasiveness, which is frequently altered in certain cancers. By reducing the AKT levels, marmelin decreases the cell survival, proliferation, and invasiveness[23].

Other phytocompounds like eugenol and citral present in *A. marmelos* has antiproliferative activities[24,25]. Chaouki *et al.*,[26]reported that citral possessed antiproliferative effects, inhibited cell cycle progression in G2/M phase, induced apoptosis of the human breast cancer cell line MCF-7 and decreased the prostaglandin E(2) synthesis.

Antiproliferative effect of *A. marmelos* revealed the fact that ethanolic extract of *A.marmelos* leaves showed significant cytotoxicity effect against MCF-7 breast cancer cells in 24 hours. The ethanolic extract (20-200µg/ml) inhibited the cell proliferation by more than 85% at its maximum concentration with an IC_{50} of 54.19 µg/ml. The results suggested that ethanolic extract of *A. marmelos* leaves inhibited the growth of MCF-7 breast cancer cells in a very effective way. Thus the observed antiproliferative effect of *A. marmelos* may be attributed to the presence of phytochemicals present in the leaves.

In conclusion, the present study reveals that *A. marmelos* leaves extract contains biologically active ingredients. In addition it possesses significant antioxidant property. The antiproliferative potential is well proven from the cytotoxicity assay. *Aegle marmelos* contains significant

antioxidants in the leaves and hence it can be used in the treatment of breast cancer and free radical mediated diseases.

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