In-vitro antiproliferative activity of M. Azedarach

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ABSTRACT

Preliminary screening of different crude extract of. *M. azedarach* was evaluated against human cancer cell lines *viz.* MCF-7 (breast cancer), SaOS-2 (osteosarcoma), and A431 (epidermoid carcinoma) to search for better herbal based anticancer agent. Solvents used were water, methanol, ethanol, n-butanol, & n-hexane. It has been found that among solvents, methanolic extract of *M. azedarach* shows comparatively superior activity suggesting presence of phytoconstituents comprising polar functionalities.

Keywords: Cancer, Antiproliferative activity, polarity Induced extraction, MTT, *M. azedarach*

INTRODUCTION

Cancer is a complex genomic disease responsible for sever ill-heath globally. The disease accounted 8.2 million deaths in 2012 & continues to swing worldwide with an estimate of 14 million new cases [1]. Expansive treatment, unaffordable medicines, drastic adverse drug reactions, limited oncological clinician, poverty, ignorance, lack of knowledge, restricted resources are some of the major roadblocks in fueling better cancer treatment thus advancing the disease towards end-up progression [2-3]. In spite of significance elucidation in carcinogenic molecular mechanism & intensive clinical trials the disease remains principal community problem globally with only three well defined & universally framed interventions (*depending upon stages*) *viz.* chemotherapy, radiotherapy, & surgery each with their own pros & cons. Chemotherapy thou a versatile global affirmative with remarkable pharmacological appurtenance however suffers limitations of non-selective targeting, sever toxicity, and tumor acquired resistance thus need of novel anticancer agent with better therapeutic profile, selectivity, specificity, and "*reach by all*" is need of present era [4].

Plants itself endowed with *self cure regimen* of phytochemicals providing immunity against pathological conditions, could equally beneficial in treating human ailments. Researcher worldwide dynamically targeted attention towards development of *phytomedicine* based anticancer agents, thus screened & studied numerous plants hence successfully pinpoint thousands or more to possess significant anticancer properties [5-10] among Meliaceae family hold distinct position [11-22]. *M. Azedarach* is an evergreen small to medium size deciduous tree in Mahogany family Meliaceae, distributed globally however specifically native to central & western china, Malaysia, Burma, & northern regions of India [23]. The plant houses essential phytochemicals responsible for multiple pharmacological activities, preclinical *in-vitro* profiling further extent its usage in treating eczema, headaches, chickenpox infection, burn, piles, gingivitis, paroxysmal fever, rheumatisim, pimples, scrofula etc. In view of above segmentation and our own intention towards development of novel herbal based anticancer agents, *in-vitro* screened *M. Azedarach* extract to three different cancer cell lines to elucidate its potential towards next generation anticancer agent.

MATERIAL & METHOD

Collection of plant material

Leaves from mature plant of *M. Azedarach* were used for this study was obtained from local milkman of District Kanpur Uttar Pradesh (India) and were characterized as per the available literature present at Institute of Pharmacy, CSJM University, Kanpur (India). The leaves were further washed thoroughly from clean distilled water, dry in shade under strict temperature control (40°C-45°C) to render any decomposition of active constituents. Once dried fully, they were milled into fine powder which is almost shady green in color.

Preparation of the extract

The powdered leafs were cold macerated in methanol for 2-weeks at room temperature with intermittent agitation. The alcoholic extract obtained was dried under reduced pressure & subjected to polarity induced solvent-solvent fractionation. The solvents were further dried under reduced pressure and crude extract so obtained were finally refrigerated for further usage.

Cell lines & culture

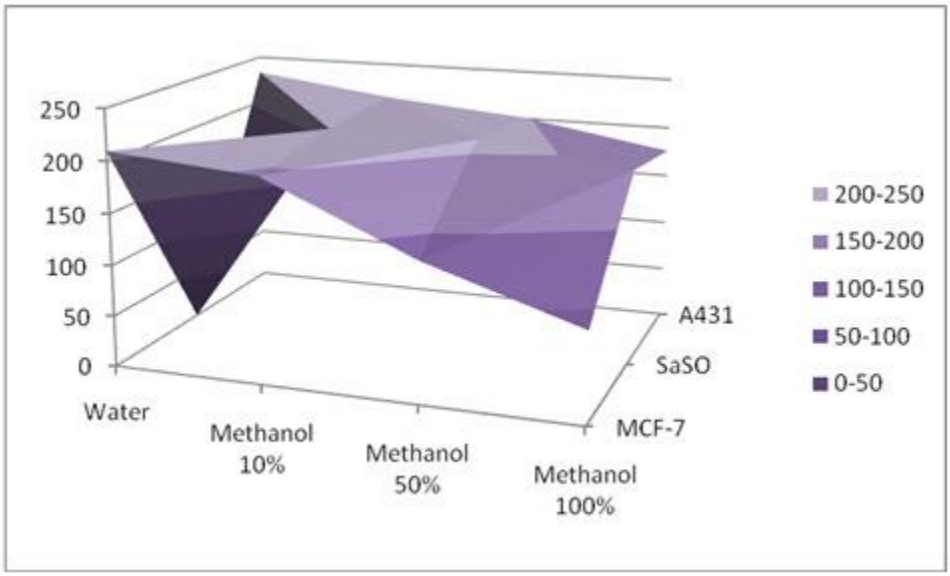
The human cancerous cell lines *viz.* MCF-7 (breast cancer), SaOS-2 (osteosarcoma), and A431 (epidermoid carcinoma) were obtained from NCCS, Pune, India. The cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM, Himedia) with 4.0 mM L-glutamine, 1.5 g/L $NaHCO_3$, 1.0 mM sodium pyruvate, 1.0 % penicillin and streptomycin solution and supplemented to contain 10 % (v/v) fetal calf serum (Himedia). Cells were grown at 37ºC, 5% CO_2 in a humidified air.

In-vitro Antiproliferative Assay

The antiproliferative activities of extracts were evaluated by MTT reduction assay as per the standard protocol[1], based on the enzymatic reduction phenomenon of MTT dye. Briefly, the cells were seeded in 100 µL complete medium in each well of 96-well culture plate for 24 hrs at 37°C in humidified, 5% CO_2 atmosphere. Stocks of leaf extract was prepared in DMSO and diluted in culture media. Further stock solutions were serially diluted in the medium to the desired concentrations and added to the wells in triplicate in such a way that the final concentration of DMSO would not exceed 0.01%. DMSO (0.01%) was used as a vehicle control. After 21 hrs of treatment, 10 µL of MTT (5 mg/mL of media without phenol red and serum) solution was added in each well and the plates were further incubated for 3 hrs at 37°C until formazan blue crystal developed. Then the supernatant was discarded from each well and 100µL of DMSO was added to solublize formazan crystals for 10-min at 37°C. The absorbance was recorded at 540 nm by a microplate reader (BIORAD-680). The percentage viability was calculated by using the formula;

% Cell viability = [(OD of control - OD of treated)/ (OD of control)] X 100

The plot of % cell viability versus sample concentration was applied to calculate the lethal concentration to 50% of the cells (IC_{50}).



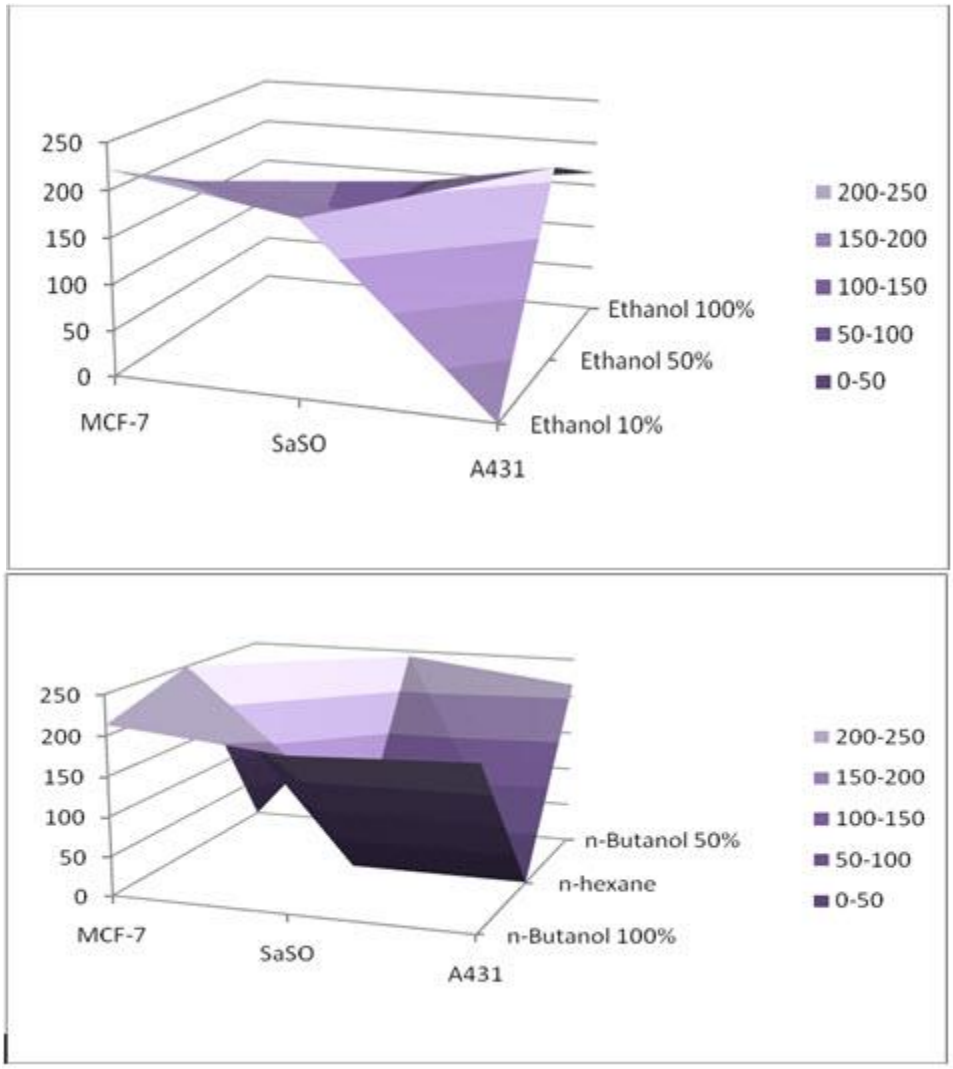


Figure 01: Antiproliferative activity of crude extract

CONCLUSION

The crude extracts were screened *in-vitro* for antiproliferative activity against MCF-7 (breast cancer), SaOS-2 (osteosarcoma), and A431 (epidermoid carcinoma) cell lines adopting MTT assay. As per American National Cancer Institute (NCI) the criteria for antiproliferative activity of crude extract is IC_{50} ≤ 30 µg/ml [24], plant extract derived from all three solvents comparatively thus shows mild antiproliferative activity (Figure-01). It was concluded that extract derived from methanol fraction shows superior activity comparatively, possibly indicating presence of polar constituents responsible for antiproliferative activity and may be used as in-future anticancer agent.

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