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ORIGINAL ARTICLE

Cytostatic activity of *Neptunia oleracea* Lour. against HL-60, OCI-LY10 and Z-138 cell lines

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ABSTRACT
Neptunia oleracea (water mimosa) is a plant consumed as a vegetable in Southeast Asia. Past works have uncovered the capability of this plant as a wellspring of common cell antioxidants. Recent investigations have revealed that N. oleracea can likewise work with soil nutrient usage and mitigate obvious N loss. In our past investigation, we illustrated the counter proliferative activity of the Neptunia oleracea Lour methanolic extricate. Further assessment was done by utilizing cell cycle examination and apoptosis tests. Presently, as an augmentation of our work, we further need to analyse the cytostatic activity of N. oleracea on HL-60, OCI-LY10, and Z-138 cell lines. Our work showed huge restraint of cell proliferation upon treatment in HL-60, OCI-LY10, and Z-138 cell lines. Inconsequential accumulation of cells in the subG0 phase of cell cycle analysis observed. Our work features the methanolic concentrate of N. oleracea as a promising lead drug to treat human promyelocytic leukemia.
Keywords: *Neptunia, water mimosa, HL-60, cell cycle, and cell proliferation.*

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INTRODUCTION

Acute Promyelocytic Leukemia (APL) is described as a blockage in cell proliferation where leukemic cells are stopped at the promyelocyte stage [1]. Portrayed by the morphology of predisposition cells (M3), t (15; 17) translocation, and coagulopathy joining scattered intravascular coagulation (DIC) and fibrinolysis. Intense Promyelocytic Leukemia is connected with essential myelofibrosis. Myelofibrosis (MF) is described by the proliferation of megakaryocytes and atypia [2].

Natural herbal products are a rich wellspring of medication, lead compounds and exhibit promise for epigenetic drug revelation [3]**.** Higenamine, Flavopiridol, Vinblastine, Vincristine are a portion of the medications affirmed by the FDA for the therapy of hematological malignancies [4]. There is a developing interest in the utilization of herbal products in the upkeep of human wellbeing. Plants contain a wide assortment of organically dynamic mixtures that are useful in the therapy of malignant growth. Over 70% of affirmed drugs are gotten, or integrated from regular items [5]. Because of the fast turn of events of drug resistive mechanisms, the disclosure of new medications is yet a need.

Neptunia oleracea is consumed as a vegetable in Southeast Asia. Past works have uncovered the capability of this plant as a wellspring of common cell antioxidants [6]. Water mimosa (*Neptunia oleracea*) has been widely identified as a feasible phytoremediator to clean up aquatic systems [7]. Recent investigations have revealed that *N. oleracea* can likewise work with soil nutrient usage and mitigate obvious N loss [8]. In our past investigation, we illustrated the counter proliferative activity of the *Neptunia oleracea* Lour methanolic extricate. Djenkolic acid and Dichrostachinic acid were bioactive constituents found in seeds of *N. oleracea* [9]. Gallic acid, Catechin, Caffeic acid, and Quercetin, a very important flavonoid antioxidant, Apigenin, and kaempferol, are accounted for to be available in the methanolic leaf concentrates of *N. oleracea*. Our essential focus in this study is to exhibit the in-vitro adequacy of *N. oleracea* in hematological cell lines using proliferation assay and cell cycle analysis.

MATERIAL AND METHODS

Assortment of plant leaves:

N. oleracea is an aquatic vegetable, a spice that is developed as a yearly sea-growing plant, coasting close to the water's edge. Plant character has been checked and affirmed from the plant information database. The leaves of plant *N. oleracea* were gathered from the Seshachalam backwoods locale of Andhra Pradesh, INDIA, confirmed with a botanist and stored at SV university herbarium (voucher number: 335). The leaves of the plant were gathered, washed with tap water to eliminate soil, and permitted to dry at room temperature for a long time. Then the dried leaves were ground into a fine powder. The acquired powder was named and utilized for the preparation of the extract.

Crude extract preparation:

The crude extracts were set up by splashing 1 gram of the dry powdered plant material in 10 mL of methanol at room temp for 48 hours and separated through Whatmann No.1 filter paper. The filtrate was additionally separated with methanol by utilizing a Soxhlet apparatus for 72 hours at a temperature not surpassing the limit of the solvent. Extracts were then kept in sterile containers, under refrigerated conditions (2-4°C).

Cell culture:

HL-60, OCI-LY10, and Z-138 leukemia cell lines were bought from American Type Culture Collection (ATCC, Manassas, VA USA). These cell lines were kept in Roswell Park Memorial Institute (RPMI) or IMDM media. The culture media was enhanced with 10% warmth inactivated Fetal bovine serum, 100 units/mL Penicillin, and 100 µg/mL streptomycin (GIBCO, USA). All the human cell lines were grown at 37 ^{0}C in a climate of 5% CO_2 in the air. The cell lines were examined for mycoplasma (Lonza, Alpharetta, GA, USA) using the maker's conditions before tests. Details of the cell lines are provided in **Table 1.**

Table 1. Cell line details

Name	Tissue	Cell Type	Disease
HL-60	peripheral blood	pro myeloblast acute	promyelocytic leukemia
OCI-LY10	tumor: DLBCL; Primary site: blood	Lymph node	B-cell lymphoma; Diffuse large B-cell lymphoma
Z-138	B cell non-Hodgkin's lymphoma	Lymphoblast	Mantle cell lymphoma

Cell proliferation assay (Resazurin):

The Cytostatic activity of plant extricates was assessed using HL-60, OCI-LY10, and Z-138 cell lines [10], [11]. Cell lines were cultivated at a density of 3000-5000 cells/well in a 96-well round-base plate, except for PBMCs which were cultivated at 1×10^5 cells/well. Doxorubicin was utilized as a positive control. Concentrate/doxorubicin was sequentially weakened to create an 8-point dose reaction. The cells were treated with the concentrate diluted in DMSO and added to the cells to accomplish the last convergences of 100, 50, 25, 12.5, 6.25, 3.25, 1.56, and 0.781 µg/mL in cells. Dose reaction of doxorubicin began at 10 µM and half log crease dilutions were performed. In control wells, the cells were treated with 0.5% DMSO. DMSO weakened mixtures were additionally diluted in culture media and added to the plates in sets of three. Plates were put in the incubator at 5% CO_2, 37 ^{0}C for 5 days.

The examination was ended using resazurin (Sigma#R7017). To the cells with media, resazurin disintegrated in PBS at a convergence of 0.1 mg/mL was added. Fluorescence was estimated at 595 nM employing Spectramax, Molecular Devices plate peruser. Fluorescence esteems were gotten from the instrument and utilized for ascertaining the rate hindrance of expansion. The vehicle-treated wells (DMSO) were utilized for standardization and considered as 0% inhibition of cell division. Percent Inhibition for treated wells was determined by utilizing the following formulae.

$$\%\,Inhibition = \frac{100 - \mathrm{RFU(sample)}}{\mathrm{RFU\,(control)}} * 100$$

*RFU- relative fluorescence unit

% Inhibition values were plotted using Graph pad prism to calculate the IC_{50}

Cell Cycle Analysis:

Cells were cultivated at a thickness of 0.5×10^6 cells/mL in a 12 well plate then treated with Extract/doxorubicin for about 24, 48 hours (3 and 30 µg/mL of concentrate and 5 µM of doxorubicin). Post-treatment, both live and dead cells were gathered and moored in chilled 70% ethanol. Cells were washed with PBS, treated with 100 µg/mL of RNase (Sigma-R6513), and afterward stained with 50µg/mL

of propidium iodide (Sigma-P4170) for 30 minutes. Samples were procured utilizing BD FACS VERSE and tests were broken down utilizing FlowJo analysis software.

Statistical Analysis:

The analysis of data was performed utilizing Graph pad prism 8 and Excel. IC_{50} was determined utilizing non-regression dose-response (hindrance/inhibition) examination utilizing Graph Pad Prism programming.

RESULTS AND DISCUSSION

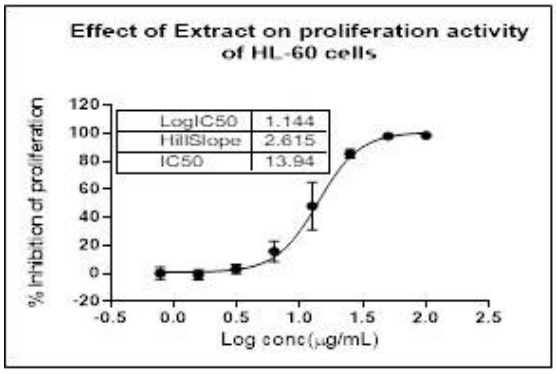
The methanolic extract of *N. oleracea* showed dose-dependent hindrance of cell multiplication in the cell lines tried. It showed an IC_{50} of 13.94, 22.92µg/mL in HL-60 and OCI-LY10 cell lines respectively **(Figure** 1). Z-138 showed, 29.1% inhibition of proliferation at 100µg/mL in Z-138 cells. No huge cytotoxicity was seen in human PBMCs with *N.oleracea* treatment as per our previous publication[12]. Our extract showed lesser harmfulness when contrasted with doxorubicin, demonstrating great selectivity.

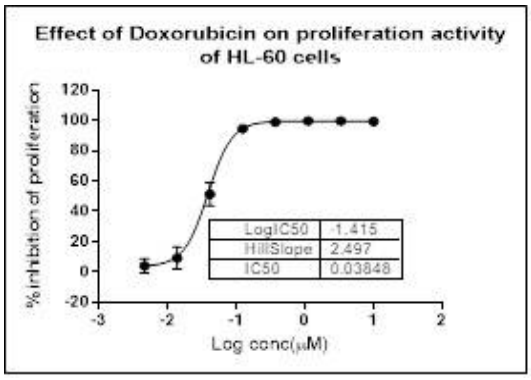
The dissemination of cells in various periods of the cell cycle upon *N.oleracea* treatment was investigated. As shown in Figure 2**,** methanolic extract treatment for 24 hours at 3 µg/mL didn't result in critical change nonetheless, 30 µg/mL treatment brought about a 12 fold increment of cells in the subG0-G1populace at 24hours. The sub G0-G1 increment peaked at 24hours and it almost remained the same till 48hours i.e.12.7 fold.

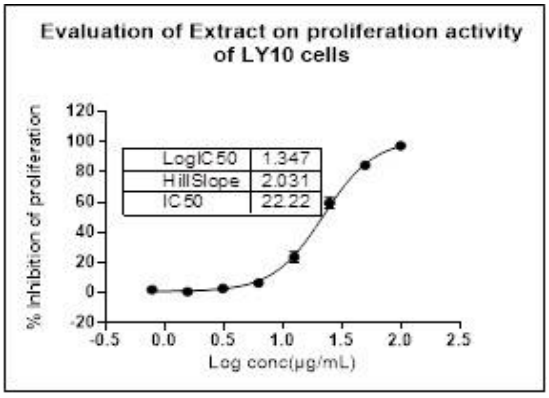
Doxorubicin treatment came about in a critical and time-dependent expansion in the subG0 populace true to form. Cell cycle investigation uncovers an expansion in the subG0 populace, showing that the activity of the extract is passed through apoptosis [13], [14].

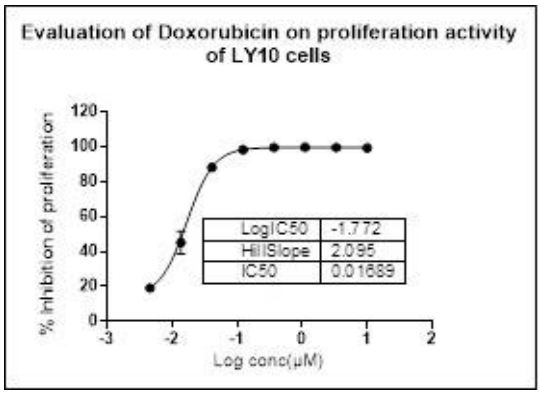
As per the new reports[6] Gallic acid, Quercitin, Kaempferol, myricetin, vitexin derivatives catechin, and caffeic acid derivatives are some of the compounds present in the methanolic extraction of *Neptunia oleracea*. Anti-cancer activity of some of these derivatives is well reported in the literature [15]. Caffeic acid is known to show anti-inflammatory and anti-cancer properties [16]. Presence of these phenolic, flavonoid compounds in the extract isolated from *Neptunia* confirms the anti-proliferative activity demonstrated in a panel of hematological cancer cell lines discussed in this publication. Increase in apoptotic and necrotic cell population observed is in line with the reported activity of Kaempferol derivatives.

Hence it establishes the fact that *N.Oleracea* leaf methanolic extract act as a cytostatic agent in Acute Promyelocytic Leukemia and DLBCL but not in mantle cell lymphoma.









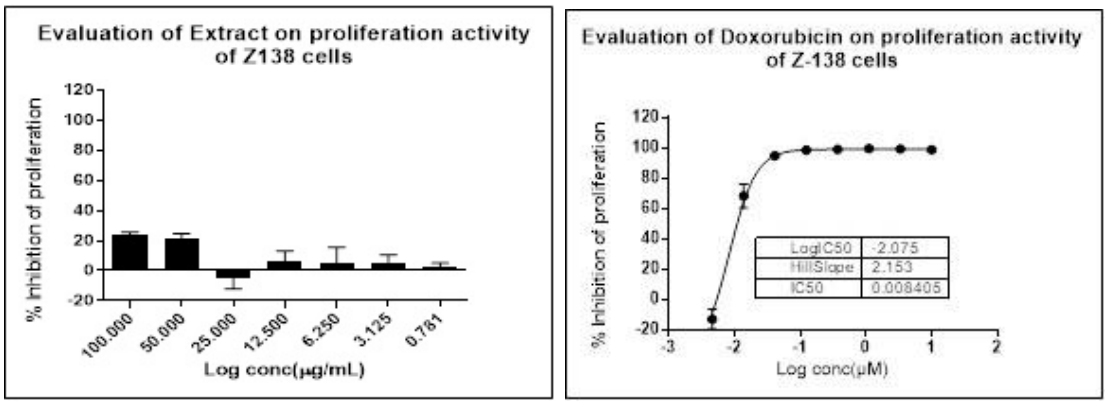
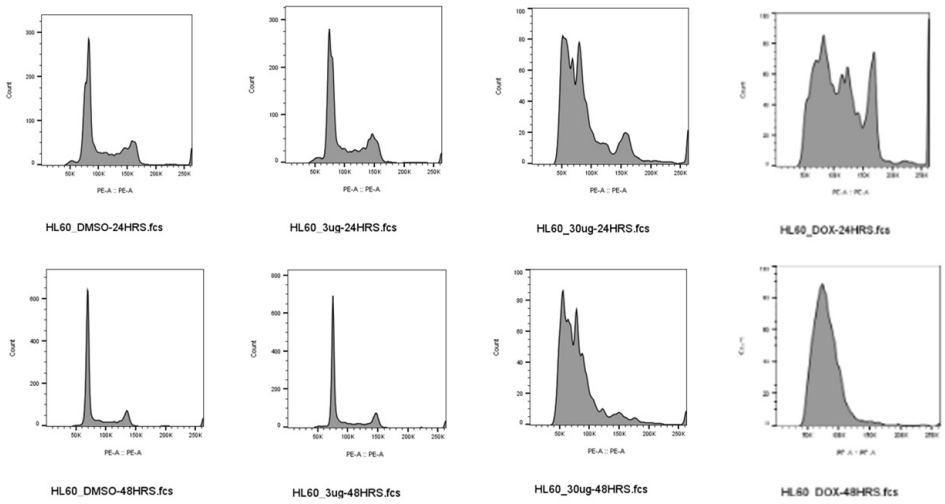
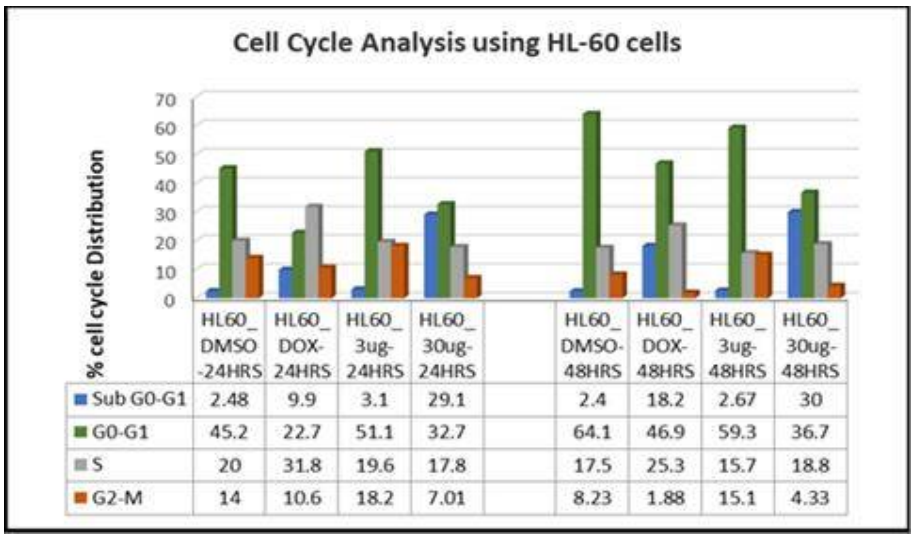


Figure 1: The cell lines were treated with extract/doxorubicin in triplicates and placed in the incubator at 5% CO2, 37°C for 5 days. Doxorubicin was used as a positive control for experiments. Post 5 days, the assay was terminated using resazurin.





	HL60_ DMSO -24HRS	HL60_ DOX-24HRS	HL60_ 3ug-24HRS	HL60_ 30ug-24HRS	HL60_ DMSO-48HRS	HL60_ DOX-48HRS	HL60_ 3ug-48HRS	HL60_ 30ug-48HRS
Sub G0-G1	2.48	9.9	3.1	29.1	2.4	18.2	2.67	30
G0-G1	45.2	22.7	51.1	32.7	64.1	46.9	59.3	36.7
S	20	31.8	19.6	17.8	17.5	25.3	15.7	18.8
G2-M	14	10.6	18.2	7.01	8.23	1.88	15.1	4.33

Figure 2: Cell cycle distribution of HL-60 cell line post *Neptunia oleracea* extract treatment. Cells were treated with extract for 24 and 48hours and fixed in 70% cold ethanol at 4°C.cells were analyzed using FACS VERSE.

CONCLUSION

N. oleracea plant is already proved as a prominent source of free radical scavengers and α-glucosidase inhibitors with phenolics as the potential candidates [17]. The present study aimed to determine the anti-

cancer properties of *N. oleracea* leaf extracts. Our outcomes propose that extract causes cell death in Leukemic disease cells without influencing normal cells. The impact of the concentrate on cell reasonability was most noteworthy in HL-60 and OCI-ly10 cells and least in Z-138.

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CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

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