

## PHYTOCHEMICAL SCREENING, IN VITRO CYTOTOXIC (AGAINST DALTON'S LYMPHOMA ASCITES CELLS) AND ANTIOXIDANT ACTIVITIES OF *MELIA DUBIA* LEAF EXTRACTS

R. Subramanian\*, Amalaraymond T., Amuprabha A., Askar Muzif A., Balaji M., Barani Ganesh S.

Ultra College of Pharmacy, Madurai, Tamil Nadu 625 107, India.

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\*Corresponding Author: R. Subramanian

Ultra College of Pharmacy, Madurai, Tamil Nadu 625 107, India.

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### ABSTRACT

*Melia dubia* (Malabar Neem) is a medicinal plant traditionally employed in the management of various ailments, although its cytotoxic potential remains inadequately characterized. This study was undertaken to investigate the phytochemical composition of *M. dubia* leaf extracts and to assess their in-vitro cytotoxic activity against Dalton's Lymphoma Ascites (DLA) cells. Fresh leaves were collected from Theni District, Tamil Nadu, authenticated, shade-dried, and subjected to sequential cold maceration using petroleum ether, methanol, and distilled water. Qualitative phytochemical screening was carried out using standard protocols, while cytotoxicity was evaluated in-vitro through the Trypan Blue Dye Exclusion Assay over a concentration range of 20–200 µg/ml, and antioxidant potential was determined using the DPPH radical scavenging assay. Phytochemical analysis revealed a variable distribution of alkaloids, flavonoids, tannins, phenolic compounds, saponins, steroids, carbohydrates, and glycosides across the three extracts. The petroleum ether extract exhibited the most pronounced cytotoxicity, producing complete (100%) cell death at 100 µg/ml, with all extracts displaying a concentration-dependent rise in cytotoxic activity. This extract also demonstrated substantial antioxidant capacity, achieving 80.2% DPPH inhibition at 100 µg/ml, corresponding to an IC<sub>50</sub> value of 62 µg/ml. These findings suggest that *M. dubia* leaf extracts, particularly the petroleum ether fraction, possess notable cytotoxic and antioxidant properties, warranting further investigation into their bioactive constituents and mechanisms of action.

**KEYWORDS:** *Melia dubia*; Cytotoxicity; Dalton's Lymphoma Ascites cells; Phytochemical screening; Antioxidant activity; DPPH assay.

## INTRODUCTION

Cancer remains a major global health burden, and although chemotherapy, radiotherapy, and surgical interventions are widely used, their clinical application may be limited by treatment-related toxicity, therapeutic resistance, and economic burden.<sup>[1,2]</sup> The chemical diversity of medicinal plants has encouraged continued investigation of plant-derived metabolites as potential sources of biologically active compounds for cancer research.

Plant-derived secondary metabolites, including alkaloids, flavonoids, phenolics, tannins, and terpenoids, have been investigated for a range of biological activities, including antioxidant and cytotoxic effects.<sup>[2]</sup> Several clinically important cytotoxic agents originated from natural products or their derivatives, including the vinca alkaloids, paclitaxel, and camptothecin-derived drugs.<sup>[3]</sup>

*Melia dubia* Cav., a member of the family Meliaceae and commonly known as Malabar Neem, is a rapidly growing deciduous tree occurring in several regions of the Indian subcontinent and Southeast Asia.<sup>[4]</sup> Traditional medicinal systems utilize various parts of *M. dubia* for treating inflammation, skin disorders, microbial infections, diabetes, and gastrointestinal ailments <sup>[4]</sup>. Phytochemical investigations have identified the presence of flavonoids, phenolic compounds, tannins, terpenoids, and alkaloids.<sup>[5]</sup> Previous studies have reported antimicrobial, antioxidant, and other pharmacological activities of *M.dubia*;<sup>[6]</sup> however, further investigation is required to clarify the relationship between its leaf phytochemical composition and cytotoxic activity.

The present study aimed to characterize the phytochemical profile of *M. dubia* leaf extracts obtained using solvents of different polarities and to evaluate their in vitro cytotoxic activity against DLA cells in relation to their phytochemical composition.

## MATERIALS AND METHODS

### Plant Collection and Authentication

Fresh leaves of *M. dubia* were collected from healthy, mature trees in Theni district, Tamil Nadu, India. Leaves were authenticated based on morphological characteristics and taxonomic descriptions by Dr. D. Stephen, Ph.D., Botanist (Retired), Department of Botany, American College, Madurai. Authenticated leaves were thoroughly washed, shade-dried at room temperature for 10-14 days, mechanically ground, and sieved (40-60 mesh) before storage in airtight containers.

### Extraction Procedure

Sequential extraction was performed using cold maceration with solvents of increasing polarity: petroleum ether (non-polar), methanol (polar organic), and distilled water (aqueous). Briefly, 50 g of dried leaf powder was successively immersed in each solvent (1:10 w/v) for 72 hours with intermittent shaking. The marc from petroleum ether extraction was used for methanol extraction, and the resultant marc for aqueous extraction. Filtrates were concentrated using a rotary vacuum evaporator and dried in a desiccator until constant weight. Extractive values were calculated as percentage w/w yield.

### Preliminary Phytochemical Screening

Test solutions (1g extract in 10 mL respective solvent) were prepared and filtered. Qualitative phytochemical tests were conducted to determine the presence or absence of selected secondary metabolites and other major phytochemical

groups: steroids (Liebermann-Burchard test), carbohydrates (Molisch's test), flavonoids (shinoda test), alkaloids (Dragendorff's reagent), glycosides (legal test), tannins (ferric chloride test), phenolic compounds (ferric chloride test), and saponins (foam test). Results were recorded as presence (+) or absence (–) of phytochemicals.

### In-Vitro Cytotoxicity Assay

Cytotoxicity was evaluated using the Trypan Blue dye exclusion method against Dalton's Lymphoma Ascites (DLA) cells according to the laboratory's standard operating procedure (SOP).<sup>[7]</sup> Test compounds (petroleum ether, methanol, and aqueous extracts) were dissolved in DMSO at concentrations ranging from 20-200 µg/ml. Viable DLA cell suspension ( $1 \times 10^6$  cells in 0.1 ml PBS) was added to tubes containing test compounds, with volume adjusted to 1 ml using phosphate buffered saline. Control tubes contained only cell suspension. Assay mixtures were incubated for 3 hours at 37°C. Following incubation, 0.1 ml of 1% Trypan Blue solution was added and incubated for 2-3 minutes. Cells were loaded onto a Neubauer hemocytometer and examined under light microscopy. Stained (dead) and unstained (live) cells were counted separately, and percentage cytotoxicity was calculated using the formula, %Cytotoxicity = (Number of dead cells / Total cells)  $\times$  100.

### Antioxidant Activity Assessment

DPPH radical scavenging activity was determined according to the laboratory's standard operating procedure (SOP), based on the DPPH free-radical scavenging method.<sup>[8]</sup> A 0.1 mM DPPH solution was prepared in ethanol. Plant extract samples (10-100 µg) were made up to 40 µl with DMSO and mixed with 2.96 ml DPPH solution. Reaction mixtures were incubated in dark conditions at room temperature for 20 minutes. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Percentage radical scavenging activity (%RSA) was calculated,

$$\%RSA = [(Absorbance\ control - Absorbance\ sample) / Absorbance\ control] \times 100$$

IC<sub>50</sub> values were determined from dose-response curves.

## RESULTS

### Extractive Yields and Phytochemical Profile

Sequential extraction of *M. dubia* leaves yielded petroleum ether (2.9% w/w), methanol (3.2% w/w), and aqueous (4.2% w/w) extracts. Preliminary phytochemical screening revealed differential distribution of secondary metabolites across extracts (Table 1). Petroleum ether extract contained alkaloids, tannins, flavonoids, saponins, steroids, and carbohydrates. Methanol extract showed presence of alkaloids, tannins, flavonoids, saponins, steroids, carbohydrates and phenolics. Aqueous extract demonstrated presence of alkaloids, flavonoids, saponins, carbohydrates, phenolics, and glycosides.

**Table 1: Preliminary phytochemical screening of *M. dubia* leaf extracts. (+) indicates presence; (–) indicates absence of phytochemicals.**

| Phytochemicals | Petroleum Ether | Methanol | Aqueous |
|----------------|-----------------|----------|---------|
| Alkaloids      | +               | +        | +       |
| Flavonoids     | +               | +        | +       |
| Tannins        | +               | +        | –       |
| Phenolics      | –               | +        | +       |
| Saponins       | –               | –        | +       |
| Steroids       | +               | +        | –       |
| Carbohydrates  | +               | +        | +       |
| Glycosides     | –               | +        | +       |

### In-Vitro Cytotoxic Activity

All *M. dubia* leaf extracts demonstrated dose-dependent cytotoxic activity against DLA cells (Table 2). Petroleum ether extract exhibited the highest cytotoxic potential, achieving 100% cell death at 100 µg/ml and maintaining this at higher concentrations. Methanol extract showed 26.3% cytotoxicity at 200 µg/ml, while aqueous extract demonstrated the lowest activity with 14.8% cell death at the highest concentration tested. At 80 µg/ml, petroleum ether extract induced 78.9% cell death, substantially exceeding methanol (10.6%) and aqueous (5.99%) extracts. The higher cytotoxic activity observed with the petroleum ether extract may be associated with the extraction of relatively non-polar constituents; however, identification and quantification of the individual active compounds are required to establish this relationship.

**Table 2: In-vitro cytotoxic activity of *M. dubia* leaf extracts against DLA cells. Values represent percentage cell death  $\pm$  standard deviation.**

| Concentration (µg/ml) | Petroleum Ether (%) | Methanol (%)   | Aqueous (%)    |
|-----------------------|---------------------|----------------|----------------|
| 20                    | 16.2 $\pm$ 2.1      | 3.6 $\pm$ 0    | 2.95 $\pm$ 0.4 |
| 40                    | 30.9 $\pm$ 1        | 5.19 $\pm$ 0.4 | 3.38 $\pm$ 0.4 |
| 60                    | 46.9 $\pm$ 0.8      | 7.42 $\pm$ 0.9 | 4.59 $\pm$ 0   |
| 80                    | 78.9 $\pm$ 2.6      | 10.6 $\pm$ 0.4 | 5.99 $\pm$ 0.5 |
| 100                   | 100 $\pm$ 0         | 14.4 $\pm$ 0.9 | 9.01 $\pm$ 1.3 |
| 200                   | 100 $\pm$ 0         | 26.3 $\pm$ 2.3 | 14.8 $\pm$ 1.4 |

### Antioxidant Activity

Petroleum ether extract demonstrated concentration-dependent DPPH radical scavenging activity, ranging from 8.8% inhibition at 10 µg/ml to 80.2% at 100 µg/ml (Table 3). The dose-response relationship exhibited strong linear regression ( $R^2 = 0.9998$ ) with IC<sub>50</sub> value of 62 µg/ml. This potent antioxidant activity indicates substantial free radical scavenging potential, which may contribute to the observed cytotoxic effects through mechanisms of oxidative stress and cellular damage.

**Table 3: DPPH radical scavenging activity of petroleum ether extract of *M. dubia* leaves.**

| Concentration (µg/ml) | DPPH Inhibition (%) | IC <sub>50</sub> Value |
|-----------------------|---------------------|------------------------|
| 10                    | 8.80%               | 62 µg/ml               |
| 25                    | 21.00%              | ( $R^2 = 0.9998$ )     |
| 50                    | 40.70%              |                        |
| 75                    | 59.60%              |                        |
| 100                   | 80.20%              |                        |

IC<sub>50</sub> = 62 µg/ml.

### DISCUSSION

This comprehensive investigation systematically evaluated phytochemical composition and in-vitro cytotoxic activity of *M. dubia* leaf extracts using multiple approaches<sup>[17]</sup>. The use of cold maceration with solvents of increasing polarity enabled selective extraction of phytochemicals with differential polarities, providing insight into the compound class distribution within the plant material.

The differential phytochemical profiles across extracts reflect the solvent polarity-dependent extraction of secondary metabolites. The relatively non-polar nature of petroleum ether favors the extraction of lipophilic constituents, which may contribute to the biological activity observed in the present study. The detection of alkaloids and tannins in the petroleum ether extract may reflect the limitations and qualitative nature of preliminary phytochemical tests; further

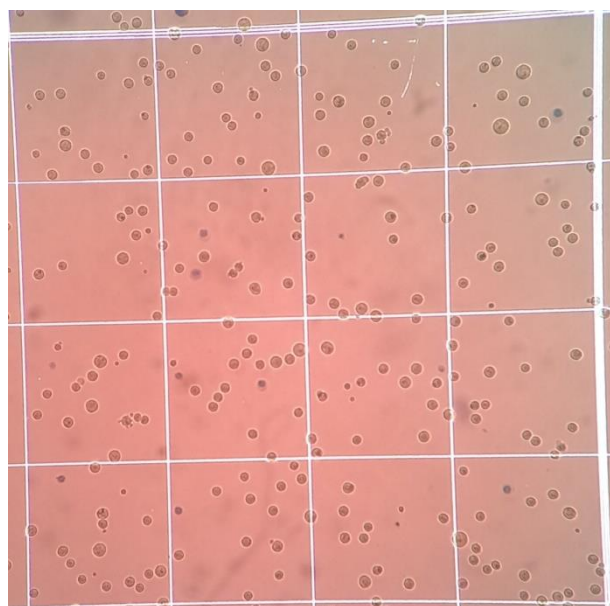
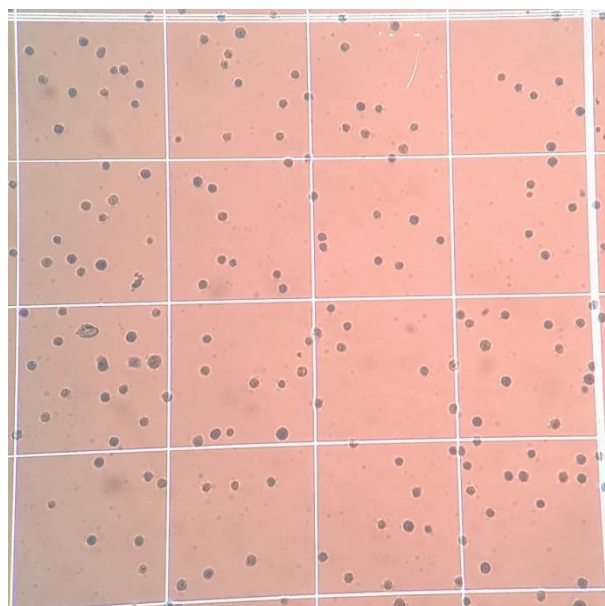
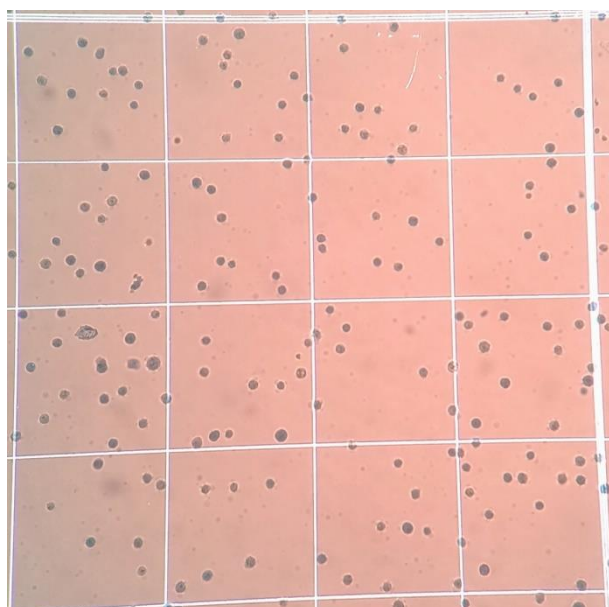
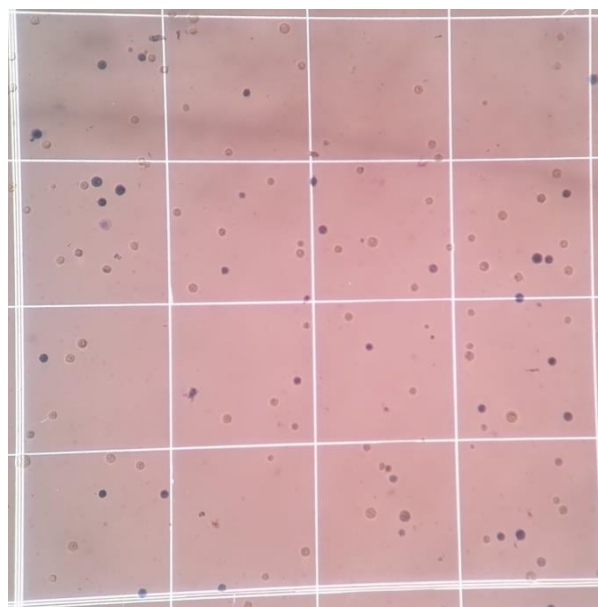
chromatographic and spectroscopic characterization would be required to confirm the identity of these constituents. Methanol's intermediate polarity enabled extraction of semi-polar phytoconstituents including phenolics, glycosides, and flavonoids. Aqueous extraction yielded water-soluble compounds including carbohydrates and some glycosides.

The superior cytotoxic activity of the petroleum ether extract compared with the methanol and aqueous extracts may be associated with the extraction of relatively non-polar constituents. This is consistent with reports on the congener *M. azedarach*, where cytotoxic activity in leaf fractions has been linked to limonoid and steroid content;<sup>[14]</sup> silver nanoparticles synthesized from *M. dubia* leaf extract itself have also shown anticancer activity against a human cancer cell line,<sup>[13]</sup> further supporting the cytotoxic relevance of this species' leaf phytochemicals." However, isolation and characterization of individual compounds are required to identify the constituents responsible for the observed cytotoxic activity. Previous studies have reported cytotoxic activities for several terpenoid compounds, suggesting that such constituents may contribute to the biological activity of plant extracts.<sup>[9]</sup> Comparable extract-level potency against DLA cells by trypan blue exclusion has been reported for other medicinal plants for example, an ethanolic stem bark extract of *Polyalthia longifolia* showed an IC<sub>50</sub> of 52.52 µg/mL against DLA cells alongside a DPPH IC<sub>50</sub> of 18.14 µg/mL.<sup>[15]</sup> Previous phytochemical investigations have reported bioactive terpenoid constituents in *M. dubia*.<sup>[10]</sup> Such constituents may contribute to the biological activity of the leaf extracts; however, compound-specific analysis would be required to establish their contribution to the cytotoxic effects observed in the present study.

The increase in cell death with increasing extract concentration indicates a concentration-dependent cytotoxic response under the conditions of the present assay. The observed concentration-dependent response supports further investigation of the cytotoxic properties of the extracts. The Trypan Blue dye exclusion assay assesses cell viability based on loss of plasma membrane integrity and therefore provides an estimate of extract-induced cell death. Notably, The antioxidant activity observed for the petroleum ether extract (IC<sub>50</sub> = 62 µg/ml) may be associated with its overall biological activity; however, a direct relationship between antioxidant activity and cytotoxicity cannot be established from the present data. A similar combination of antioxidant and DLA-cytotoxic activity has been reported for *Garcinia morella* extracts, where the anticancer effect was subsequently attributed to caspase-mediated apoptosis rather than antioxidant action alone,<sup>[16]</sup> underscoring why compound-level studies are needed before drawing a mechanistic link in the present extracts. The observed antioxidant activity may be associated with the biological effects of the extract; however, the molecular mechanisms underlying the cytotoxic activity were not investigated in the present study. The detection of steroids in the petroleum ether extract provides a possible basis for further investigation, although their specific contribution to the observed cytotoxic activity remains to be established.

While methanol and aqueous extracts showed lower cytotoxic activity. Although the methanol and aqueous extracts exhibited lower cytotoxic activity, their phytochemical profiles included phenolics, flavonoids, and glycosides. The contribution of these constituent classes to the observed activity requires further investigation. albeit at concentrations requiring further optimization.

Limitations of the present study include restriction to in-vitro assessment using a single cancer cell line; evaluation in additional cancer cell lines (breast, colon, liver) would strengthen conclusions regarding selectivity and broad applicability. Further investigation via isolation and structural characterization of active compounds, elucidation of molecular mechanisms of action, and in-vivo efficacy studies would establish the therapeutic potential more comprehensively.

**100% LIVE CONTROL****14% AQUEOUS EXTRACT****26% METHANOL EXTRACT****100% PET-ETHER EXTRACT**

Images that obtained from the in-vitro cytotoxic activity test

## CONCLUSION

This study provides preliminary experimental evidence of cytotoxic and antioxidant activities in *M. dubia* leaf extracts. The petroleum ether extract exhibited superior cytotoxic activity against DLA cells, with 100% cell death at **100  $\mu\text{g/ml}$**  concentration and  **$\text{IC}_{50} = 62 \mu\text{g/ml}$**  for antioxidant activity. The observed biological activities occurred alongside the detection of several phytochemical classes, including steroids, alkaloids, and flavonoids.

The findings suggest that *M.dubia* leaves, particularly the petroleum ether extract, may contain bioactive constituents with cytotoxic and antioxidant properties. Further studies involving compound isolation, mechanistic investigations, additional cancer cell lines, toxicity evaluation, and in vivo models are necessary to determine their therapeutic relevance. However, further research including isolation and characterization of active principles, investigation of

molecular mechanisms of action in multiple cancer cell lines, toxicity profiling, and in-vivo efficacy studies in animal tumor models are recommended to advance development toward clinical evaluation. These findings support further phytochemical and pharmacological investigation of *M. dubia* as a potential source of bioactive compounds for cancer research.

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