

Research Article



Antioxidant Activity and In-vitro Cytotoxicity Study of Novel *Dombeya wallichii* (An Invasive Plant) against Human Lung Cancer Cell Line

Deepak Kumar U*, Nivetha L, Devipriya @ Nisha P

*Department of Biotechnology, PSG College of Arts & Science, Coimbatore, India.

*Corresponding author's E-mail: deepak.udhayakumar@outlook.in

Received: 22-05-2021; Revised: 24-07-2021; Accepted: 30-07-2021; Published on: 15-08-2021.

ABSTRACT

Recent research is focusing on the search for new types of natural chemotherapeutic agents derived from plants which are proving to be excellent sources of new compounds. The present research article was aimed to study the antioxidant activity of ethanolic extracts of *Dombeya wallichii*, which is an invasive plant (Plants that do not occur naturally in a region but proliferate in the area they have been introduced into) by DPPH radical scavenging method which exhibited antioxidant activity with IC₅₀ value of 744.04 µg/ml. The cytotoxic activity of ethanolic extracts from the leaves of *Dombeya wallichii*, by in-vitro cytotoxic assays like MTT against lung adenocarcinoma epithelial cell line A549 which exhibited anticancer activity with IC₅₀ value of 2.50 µg/ml concentration. This study creates the awareness about this plant which is having potential antioxidant activity, cytotoxic activity and the outcomes propose that *D. wallichii* as a potential source of alternative medication drugs for treating cancer. Further research is required to find out the effective mechanisms responsible for anticancer properties and for curating the therapeutic benefits of the less explored and exploited invasive species.

Keywords: Invasive plants, *Dombeya wallichii*, Antioxidant activity, cytotoxic, MTT assay, A-549 cell line.

QUICK RESPONSE CODE →

DOI:
10.47583/ijpsrr.2021.v69i02.015



DOI link: <http://dx.doi.org/10.47583/ijpsrr.2021.v69i02.015>

INTRODUCTION

Invasive plants are exotic or foreign or non-native plants, that has been introduced by humans intentionally through humans or accidentally from one region to a different.¹ About 40% of the species within the Indian flora are alien (non-native), of which 25% are invasive.² Invasive plants are being incorporated into everyday usage by indigenous traditional people as food and fuel. More importantly, some invasive are being harvested for its perceived medicinal properties. In Republic of South Africa, various studies revealed that invasive plants are utilized by communities to treat different ailments. Example, *Bidens pilosa*, is reported to own antimicrobial, anti-helminthic, anti-malarial and anti-ulcerogenic properties.³

Medicinal plants are potential source of raw materials used in the manufacture of drugs and perfumery products. More than a quarter of all the medicines used in the world today contain natural compounds derived from plants that often serve lead molecules whose activities can be enhanced by manipulation through combinations with chemicals and by synthetic chemistry that can be exploited in the field of new drugs research and development.⁴

Medicinal plants are considered to be the exclusive source of life saving drugs for majority of world's population and the potential of using the natural products as anti-cancer drugs was recognized in 1950's by U.S Natural Cancer Institute (NCI) and major contributions have taken for the discovery of naturally occurring anti-cancer drugs.

The genus *Dombeya* is named after Joseph Dombey, an 18th-century French botanist, doctor, and explorer.⁵ *Dombeya wallichii* is often referred to as Pink Ball tree, because of the fragrant, showy, clusters of pink ball flowers which are 4 - 6 inches across. *Dombeya wallichii* is a large shrub with attractive large heart shaped velvety leaves. Aphids, nematodes are minor problems for pinkball and no diseases are of major concern.⁶ *Dombeya wallichii* was used as Stomach medicine by Akha hill tribes of Northern Thailand in early 1980's.⁷ *Dombeya wallichii* is an invasive plant, and hence no prominent research studies on this plant have been noted in the past. Here we investigated preliminary qualitative phytochemical analysis, antioxidant activity by DPPH radical scavenging method and cytotoxic activities in cancer-derived lung adenocarcinoma epithelial cell line (A549) using ethanolic leaf extract of *Dombeya wallichii*.

MATERIALS AND METHODS

Plant sample collection and authentication

Fresh leaves of *Dombeya wallichii* were collected from Nehru Park, Kotagiri, The Nilgiris, Tamil Nadu. The plant was authenticated (Specimens No. BSI/SRC/5/23/2021/Tech/326) at Botanical Survey of India (BSI), Coimbatore, Tamil Nadu.



Preparation of leaves extract

The fresh leaves were collected and washed under running tap water to remove all debris, and the collected leaves were shade dried. The dried plant material was subjected for the Soxhlet extraction. 30g of the plant material was taken to fill the porous cellulose thimble. Following this, the solvent (250 ml of ethanol) was added to a round bottom flask, which was attached to a Soxhlet extractor and condenser on an isomantle. The crushed plant material was loaded into the thimble. The solvent was heated using the isomantle and begin to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it poured back to the flask and the cycle begins again. The process continued for a total of 16 hours. Once the process has finished, the ethanol was evaporated using a rotary evaporator, leaving an extracted plant material (about 2 to 3 ml) in the glass bottom flask, and the crude extract was obtained.

Phytochemical screening analyses

Preliminary phytochemical screening was carried out to determine the presence of various bioactive constituents like Alkaloids, anthraquinones, carbohydrates, coumarins, flavonoids, steroids and terpenoids in the ethanolic crude extract by using standard methods.⁸

Antioxidant activity

The antioxidant activity of *D. wallichii* extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the standard method.⁹ The ethanolic leaf extract (0.1 ml) was added to 3ml of a 0.004% ethanol solution of DPPH. Absorbance at 517nm was determined after 30 min, and the percentage inhibition activity was calculated from $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. The inhibition curves were prepared and IC_{50} values were obtained.

Cytotoxicity assay

In vitro cytotoxicity test method was performed in ethanolic leaf extract. The cells were grown in MEM medium supplemented with 10% FBS. The cells were trypsinized and counted on cell hemocytometer. Approximately 10,000 cells per well were seeded in a 96-well plate and incubated for 24 hours. The culture medium from the A549 cells was replaced with fresh medium. The samples at different concentrations were added in triplicates on the cells. After incubation at $37 \pm 1^\circ C$ for 18 h, MTT (1mg/1ml) were added in all the wells and incubated for 4 h. After incubation, DMSO were added in the wells and read at 570 nm using photometer. Cytotoxicity and cell viability were calculated by using formula.

Cytotoxicity = $[(Control - Treated) / Control] \times 100$

Cell viability = $(Treated / Control) \times 100$

RESULTS AND DISCUSSION

Phytochemical screening analysis

The extracts contained many chemical constituents like alkaloids, carbohydrates, terpenoids, coumarins, flavonoids. These chemical constituents are called as secondary metabolites and are responsible for therapeutic effects.¹⁰ Preliminary qualitative phytochemical analysis were performed, and the compounds like Alkaloids, carbohydrates, coumarins, flavonoids and terpenoids were present in ethanolic extract of *Dombeya wallichii*. To confirm the presence or absence of these secondary metabolites, ethanolic extracts were subjected to coloured reactions of chemical tests.¹¹ Flavonoids and are the largest classes of plant phenolics and are known to have good antioxidant activity both in vitro and in vivo reported that flavonoids may preserve beta-cell function by reducing oxidative stress- induced tissue damage.¹²

Antioxidant activity

DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. Ascorbic acid was chosen as the reference antioxidant for this test. The highest scavenging obtained was 71.8% at 1000 $\mu g/ml$. Scavenging of DPPH radical was found to rise with increasing concentration of the extracts from 200-1000 $\mu g/ml$ (Figure 1). *D. wallichii* extract exhibited remarkable antioxidant activity with the IC_{50} value of 744.04 $\mu g/ml$.

Antioxidants fight against free radicals and protecting the body from different diseases. Oxidative damage can be overcome by many synthetic drugs available but these drugs are associated with adverse side effects.¹³ The plant-derived antioxidants include ascorbic acid, carotenoids and phenolic compounds.¹⁴ These antioxidant activities of plants may act by preventing the production of free radicals or by scavenging free radicals produced in the body.

Cytotoxicity assay

Cancer is a disease that has always been a major threat and has been characterized by proliferation of abnormal cells. Though Chemotherapy is now being used as a standard treatment method, search for anticancer agents from natural products has increased. In order to annotate the mechanism of prevention of cancer and to identify new anticancer activities a number of plants have been explored.¹⁵

In the present study the cytotoxic effect of ethanolic extracts of *Dombeya wallichii* on the growth of A549 cell line were examined by the MTT assay. The leaf extract showed moderate to severe cytotoxic reactivity to A549 cells after 24hr contact. The maximum percentage of inhibition was found to be 75.1% at 100 $\mu g/ml$ (Figure 2). Control gave none cytotoxic reactivity as expected. Phase contrast microscopic images of A549 cells are given (Figure 3). The ethanolic extract exhibited remarkable activity with



the IC₅₀ value of 2.50 µg/ml. Ethanolic leaf extract showed in-vitro cytotoxicity activity against Lung cancer cell line. These results suggest that it may be an attractive option for the “drug hunters” as a potential agent for the

management of human cancer and these activities may be due to either of the presence of antioxidants such as vitamin C, anthocyanin, folate carotenoids.¹⁶

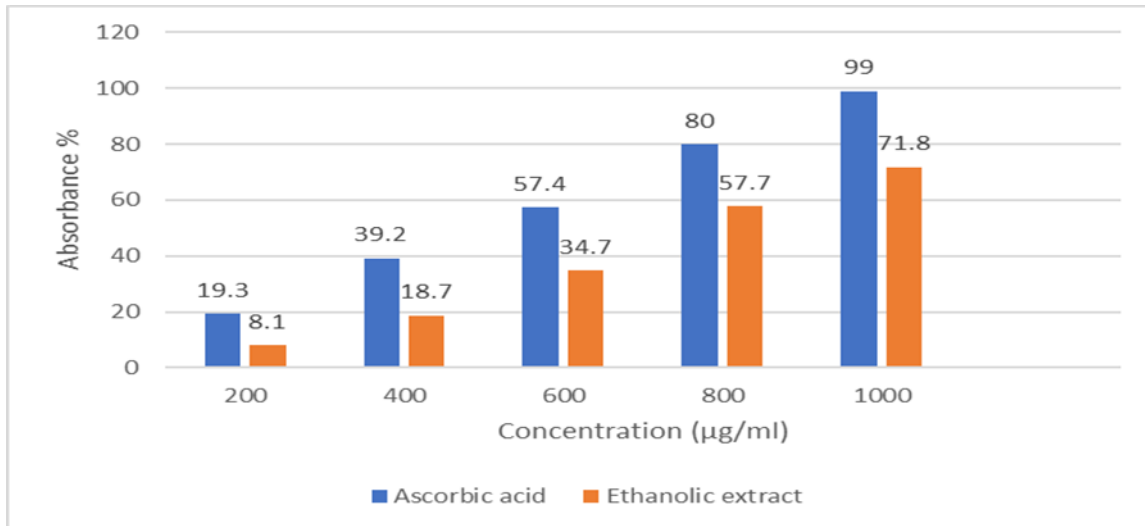


Figure 1: Total antioxidant activity of ethanolic extract of *Dombeya wallichii*.

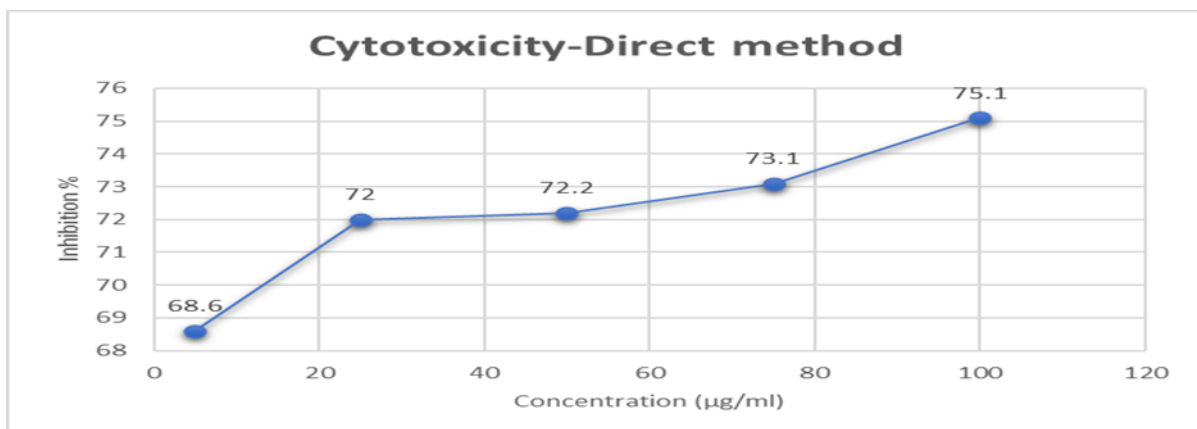


Figure 2: Cytotoxicity effect of ethanolic extract of *D. wallichii* as determined by MTT assay.

Cytotoxicity – Direct Method

Cell line: A549

Sample particulars: *Dombeya wallichii*

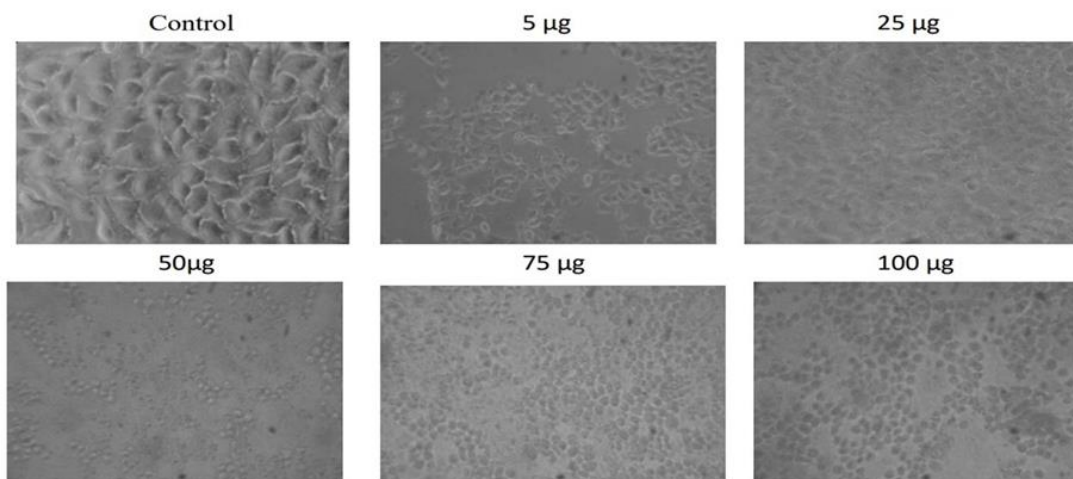


Figure 3: Phase contrast microscopy image of A549 cells treated with ethanolic leaf extract of *D. wallichii* for 24 h.

CONCLUSION

In conclusion, the present study demonstrated that *Dombeya wallichii* leaf extracts contain several secondary metabolites that can be further studied for their therapeutic purposes. *D. wallichii* showed high antioxidant activity and exhibited strong anticancer activity on lung cancer (A549) cell line. Our results provide the basis for the further investigation and potential identification of medicinal compounds of anticancer property. From this observation, it was clear that the ethanolic leaf extract of *D. wallichii* showed potential anticancer activity and the possibilities for the herbal treatment of these deadly diseases and this study could be continued to explore therapeutic activities other than cytotoxicity and antioxidant activity in leaves as well as other parts of the plant.

REFERENCES

- Reddy CS. Catalogue of invasive alien flora of India. Life Sci J. 2008;5(2):84–9.
- A.S R, L.C R, J.P G, Singh.J.S. Invasive alien species and biodiversity in India. Curr Sci. 2005;88(4):539–40.
- Maema LP, Potgieter M, Mahlo SM. Invasive alien plant species used for the treatment of various diseases in limpopo province, South Africa. African J Tradit Complement Altern Med. 2016;13(4):223–31.
- Jayalakshmi B, Raveesha KA, Amruthesh KN. Phytochemical investigations and antibacterial activity of some medicinal plants against pathogenic bacteria. J Appl Pharm Sci. 2011;1(5):124–8.
- Carter BK. Tropical Hydrangea *Dombeya wallichii*. 2009;1–2.
- Gilman EF, Watson DG. *Dombeya wallichii*. 1993;(11):1–3.
- Anderson EF. Ethnobotany of Hill Tribes of Northern Thailand . I. Medicinal Plants of Akha1. 2016;40(1):38–53.
- Jeenu, Joseph L, George M, Bindhu AR. World Journal of Pharmaceutical Research Phytochemical Screening And Antioxidant Activity Of Various Extracts Of Clerodendru M Paniculatum Linn. 2018;7(13):555–68.
- Zaman KA, Khalid AA. Free radical scavenging activity of some bangladeshi medicinal plants. Pharmacologyonline. 2015;3(12):29–32.
- Padmalochana K, Rajan MSD. Antimicrobial Activity of Aqueous, Ethanol and Acetone Extracts of Sesbania grandiflora Leaves and Its Phytochemical Characterization. Int J Pharma Sci Res. 2014;5(12):957–62.
- Aa A, Pb K, Pattiyappan S, Ra U, Peo R. Phytochemical Screening of Sesbania grandiflora (Linn). Int J Biosci Nanosci. 2014;1(2):2014.
- Kasote DM, Katyare SS, Hegde M V., Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci. 2015;11(8):982–91.
- Suwalsky M, Avello M. Antioxidant capacity of Ugni molinae fruit extract on human erythrocytes: An in vitro study. J Membr Biol. 2014;247(8):703–12.
- Lupea AX, Pop M, Cacic S. Structure-radical scavenging activity relationships of flavonoids from Zziphus and Hdrangea extracts. Rev Chim. 2008;59(3):309–13.
- Uma Devi P, Selvi S, Devipriya D, Murugan S, Suja S. Antitumor and antimicrobial activities and inhibition of in-vitro lipid peroxidation by *Dendrobium nobile*. African J Biotechnol. 2009;8(10):2289–93.
- Bhavna M, Keyur H. D, Dharmik D. P, Pinal D. P, Divya P, Maulik P. S, et al. In-vitro cytotoxicity evaluation of aqueous fruit and leaf extracts of *Grewia asiatica* using MTT Assay. Der Pharma Chem. 2011;3(3):282–7.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

For any question relates to this article, please reach us at: editor@globalresearchonline.net

New manuscripts for publication can be submitted at: submit@globalresearchonline.net and submit_ijpsrr@rediffmail.com

