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Cytotoxic and antiproliferative activities of Berberis aristata hydroethanolic extract against human lung cancer cell line

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Abstract
The present research aimed to examine the effectiveness of *Berberis aristata* DC. hydroethanolic extract in human lung cancer cell lines A549. The cytotoxicity assay and cell viability were investigated using the cytotoxicity assay performed at the Department of Biosciences, Sardar Patel University, Gujarat.
The cytotoxicity of *B. aristata* DC. crude extract investigated against lung adenocarcinoma cell lines A549 gave an IC_{50} value of 47.84 ± 0.49 (µg/ml), indicating its effectiveness as a potential anti-cancer agent. Notable changes in the morphology of the selected cell lines were also noted. The MTT assay revealed that the *Berberis aristata* DC extract demonstrates significant cytotoxicity against A549 cells, suggesting a potential anti-cancer agent.

Keywords: *Berberis aristata* DC., Lung cancer, A549

Introduction
Lung cancer remains one of the leading causes of cancer-related mortality worldwide, accounting for approximately 18% of all cancer deaths (Sung *et al.*, 2021) [21]. Among the different types of lung cancer, non-small cell lung carcinoma (NSCLC), including adenocarcinoma (A549 cell line), constitutes nearly 85% of cases (Molina *et al.*, 2008) [10]. Despite advancements in chemotherapy and targeted therapies, the high recurrence rate and drug resistance in lung cancer necessitate the exploration of novel therapeutic agents, particularly from natural sources.
Medicinal plants have long been utilized in traditional medicine for their diverse pharmacological activities, including anti-cancer properties. *Berberis aristata* DC., commonly known as Indian Barberry, is widely used in Ayurvedic and Unani medicine for its antimicrobial, anti-inflammatory, and antioxidant effects (Singh *et al.*, 2010) [18]. The plant is rich in bioactive alkaloids, particularly berberine, which has been reported to exhibit significant anti-cancer properties through mechanisms such as apoptosis induction, cell cycle arrest, and inhibition of tumor proliferation (Imenshahidi & Hosseinzadeh, 2019) [5]. However, the cytotoxic effects of the crude hydroethanolic extract of *B. aristata* on lung adenocarcinoma (A549) cells remain unexamined.
The present study aims to investigate the cytotoxic potential of *Berberis aristata* DC. hydroethanolic extract against human lung cancer cell lines (A549). The cytotoxicity assay, performed using the MTT method, evaluates the inhibitory concentration (IC50) and morphological changes in treated cancer cells. Understanding the potential anti-cancer effects of *B. aristata* may provide valuable insights into its use as a complementary therapeutic agent in lung cancer treatment.

Phytochemical studies
The *Berberis aristata* DC. plant is rich in various chemical constituents, predominantly alkaloids such as protoberberine and bisbenzylisoquinoline. The plant contains berberine, oxy berberine, berbamine, aromoline, karachine, palmatine, oxyacanthine, and taxilamine. It contains protoberberine and bis isoquinoline type of alkaloid (Chadha, 1948) [2]. The root of the plant contains alkaloids, which are berbamine, berberine, oxycanthine, epiberberine, palmatine, dehydrocaroline, jatrorhizine, karachine, dihyrokarachine, taximaline, oxyberberine, aromoline, and columbamine. Various polyphenolic flavonoids like caffeic acid, quercetin, chlorogenic acid, keratin, and rutin are reported from the flower extract of *Berberis aristata* DC. (Sivakumar *et al.*,1991) [19].

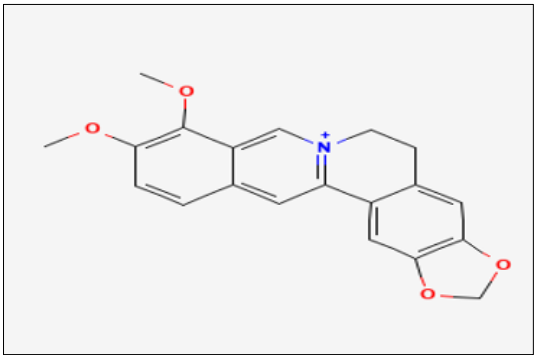


Fig 1: Molecular structure of 'Berberine' (PubChem. ncbi.)

Anticancer activity

Berberine, derived from *Berberis aristata* DC., is a potent agent utilized in anticancer therapy (Neag, *et al.*, 2018) [12]. It has demonstrated anticancer properties against various types of cancer-like breast, colon, cervical, kidney, liver, and lung cancers (Rokade *et al.*, 2022) [16].

Materials and Methodology

The medicinal plant *Berberis aristata* DC. has been chosen for study and collected Indo-Myanmar Friendship Road, Imphal, Manipur, India.

Types of equipment needed for work: Weighing Balance. Absolute Ethyl Alcohol. Filter Papers. Glass bottles. Petri Plates (Large and Small). Soxhlet extractor. Beakers. Spatula. Conical Flasks. Funnel. Amber glass bottles. Refrigerator.

Methods of Plant Sample Collection

The sample was collected from healthy plants. The stem of the plant was cut along with the bark and chopped into fine pieces. The material was dried completely for 12- 15 days in the shed. Then the pieces were ground into powder. The powder was sieved properly, removing all the debris. Lastly, the fine powder was packed in airtight pouches.

Procedure of Plant Extraction Preparation

A 100-gram quantity of *Barberis aristata* DC. stem and bark powder were accurately weighed and subjected to a cold maceration process. The plant material was immersed in one Liter of absolute ethyl alcohol in two separate glass bottles, each containing 500 ml of absolute alcohol and 50g of plant material. The bottles were sealed and shaken vigorously for several minutes before being stored in a dark location. The mixture was continuously agitated two to three times a day for a period of three to four days. Following filtration through filter paper, the extract was concentrated using a Soxhlet extractor and then poured into Petri dishes for room-temperature evaporation over two to three days. The extract was collected from petri plates with the help of proper sterilized equipment. The final product was transferred to amber glass bottles, labeled, and stored in a refrigerator.

MTT assay

Cells were seeded in a 96-well flat-bottom microtiter plate at a density of 1 × 104 cells/well and allowed to adhere for 24 hours at 37°C in a CO_2 incubator. After 24 hours of incubation, the culture medium was replaced with fresh medium. Cells were then treated with various concentrations of the desired compound for 24 hours at 37°C in a CO2 incubator. After 24 hours of incubation, the culture medium was replaced with fresh medium. Subsequently, 10 µl of MTT working solution (5 mg/mL in phosphate buffer solution) was added to each well, and the plate was incubated for 4 hours at 37°C in a CO_2 incubator. The medium was then aspirated, and the formed formazan crystals were solubilized by adding 50 µl of DMSO per well for 30 min at 37°C in a CO_2 incubator. Finally, the intensity of the dissolved formazan crystals (purple color) was quantified using the ELISA plate reader at 540 nm. This assay for assessing cytotoxicity and cell viability was performed at the Department of Biosciences, Sardar Patel University, Gujarat.

Results and Discussion

The MTT assay revealed that the *Berberis aristata* DC. extract significantly decreased the viability of A549 cells, indicating a notable cytotoxic effect by MTT assay (Table 01). The IC_{50} values obtained, about 47.84 ± 0.49, represent the mean standard error for the assay performed in triplicate with n = 3. To evaluate the influence of *Berberis aristata* DC. extract from lung cancer cells were compound the treated and untreated cell lines using a phase contrast microscope. The results demonstrated that lung cancer cells exposed to the extract underwent substantial alterations in their morphology, appearing markedly 41 rounder compared to the untreated cells (Figures A and B). The concentration-response graph of percentage viability in A549 cells treated with *Berberis aristata* DC. extract is shown below.

Table 1: Effect of *Berberis aristata* DC. crude extract on the morphology of A549 cells

Sr. No.	Compound Name	Cell Line	IC50 values (µg/ml)
01	BA01	A549	47.84 ± 0.49

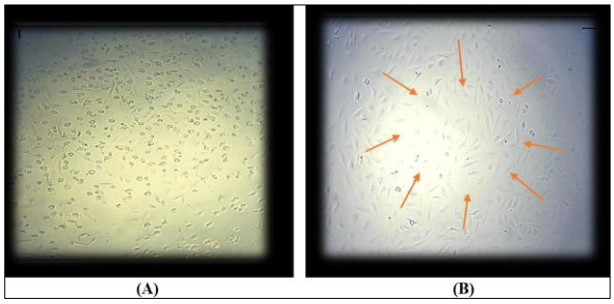
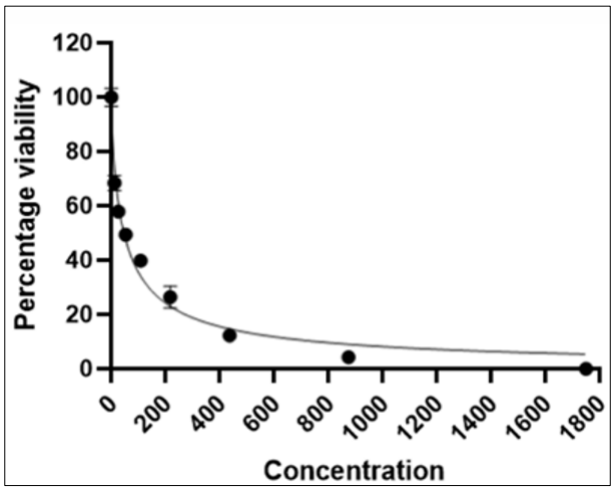


Fig 2: (A) photograph indicates the normal morphology of the A549 cell and (B) Image of A549 cells treated extract. After 24h incubation photograph. Arrows indicate the changes in cell morphology



Graph 1: Effect of Berberis aristata DC. on human lung adenocarcinoma

Conclusion

The cytotoxicity of *B. aristata* DC. crude extract against lung adenocarcinoma cells (A549) gave an IC50 value of 47.84 ± 0.49 µg/ml, indicating its effectiveness as a potential anti-cancer agent.

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