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# Anti-Lung Cancer Activity of the Herbal Medicinal Plant *Cycas revoluta*

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# Anti-Lung Cancer Activity of the Herbal Medicinal Plant *Cycas revoluta*

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**Abstract**

**Background: Phytochemicals have demonstrated strong potential in cancer therapy, and several researchers became interested in evaluating the anti-cancer effects of *Cycas revoluta*, a plant in herbal medicine.**

**Objectives: The present study examined the anti-proliferative effect of the n-butanol fraction from *Cycas revoluta* root extract on A549 human lung adenocarcinoma cells.**

**Materials and Methods: The roots were defatted with n-hexane and then extracted. The reflux technique—Soxhlet extraction with 85% ethanol and 15% distilled water—was then used to obtain the crude extract of the root, which was subsequently fractionated using the solvents chloroform, ethyl acetate, and n-butanol, respectively. Cytotoxicity was evaluated using the MTT assay after 72 hours of treatment.**

**Results: Initial results indicate that the n-butanol fraction has potential as an anti-cancer agent for lung cancer cells and merits further investigation into its mechanisms and active compounds. The present work adds to the increasing evidence of plant-based anti-cancer agents and the potential of *C. revoluta* in lung cancer therapy.**

**Conclusion: The n-butanol extract from the roots of *C. revoluta* exhibits notable anti-proliferative effects on A549 lung cancer cells, indicating its potential as a natural anti-cancer therapeutic agent. Additional research is required to identify the active components, clarify the molecular mechanisms involved, and assess the in vivo effectiveness.**

***Keywords:*** Lung cancer, *Cycas revolute*, Anti-cancer activity, A549 cells, MTT assay

## 1. Introduction

In 2020, lung cancer accounted for more fatalities than cancers of the prostate, colon, and breast put together, making it the most prevalent cause of cancer death and the number two cancer diagnosis for both sexes [1]. Throughout the United States, this condition is currently the second most frequently diagnosed non-skin cancer in both men and women. It was anticipated that there would be about 155,870 lung cancer-related deaths and 222,500 new cases in the US in 2017. It accouns for around 13% of newly diagnosed cancer cases and 26% of deaths caused by cancer in the US [2].

This disorder was sporadic before the 20th century. Moreover, since this cancer was initially described in the 18th century by Giovanni Battista Morgagni, it was difficult to diagnose for years. By 1898, 140 cases had been reported in worldwide medical literature, and Adler found records of only 374 instances when he wrote his review in 1912 [3]. Although initial cases of lung cancer were primarily noted among miners and certain other occupational groups in the nineteenth century, a significant rise in lung cancer cases was recorded in the early twentieth century. Since then, lung cancer has become the most common cancer in males in many nations, making it the world's top cause of cancer-related death. [4] Additionally, patients who have this deadly disease typically have a bad prognosis [5]. Although advances in targeted therapy and immunotherapy have improved outcomes in non-small cell lung cancer, the overall cure and survival rates remain low, particularly in metastatic disease, underscoring the urgent

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need for new, more effective treatments [6] Historically, plants have served as fundamental sources for discovering natural products used in medicine [7]. Numerous compounds found in plants, such as paclitaxel, etoposide, docetaxel, topotecan, vincristine, and irinotecan, are recognized as the most efficient chemotherapeutic agents currently accessible [8]. Among these, Cycas *revoluta*, a plant traditionally used in folk medicine, has attracted growing scientific interest. Phytochemical investigations have identified a range of bioactive compounds, particularly flavonoids and biflavonoids, in its leaves. In addition, several studies have demonstrated cytotoxic and anticancer properties of its extracts in vitro [9–11]. This plant was one of the most significant plants widely used in traditional herbal medicine for functional disorders such as urinary problems, neuralgia, and malignant ulcers [12]. A minimum of 68 chemical compounds have been extracted from various parts of *C. revoluta*, including leaves, seeds, pollen grains, male cones, and female cones. The components include 25 flavonoids, 15 nonprotein amino acids, nine glucosides, eight fatty acids, three benzenoids, two terpenes, one amino acid, one diterpenoid, one triterpenoid, one sterol, one ester, and one steroid. [13].

Numerous studies on the *Cycas revoluta* plant have demonstrated its efficacy against various cancer types. A study indicated that the extract of *Cycas revoluta* Thunb selectively targeted and eliminated gastric cancer cells, exhibiting a reduced effect on normal gastric cells. This extract greatly hampered the development, movement, and invasion of stomach cancer cells [14]. Additionally, a study indicates that the *Cycas revoluta* methanolic extract cone suggests that this extract may serve as a potential candidate for colon cancer protection [12]. This research investigated the inhibitory effect of the n-butanol fraction of *Cycas revoluta* root against lung cancer cells to see the extract's effectiveness.

## 2. Materials and methods

### *2.1. Collection and extraction of cycas revoluta root*

*Cycas revoluta* Thunb from the Cycadaceae family is documented by Dr. Zainab Abd-oun Ali (Biology Department/College of Sciences/ Baghdad University), after which the entire plant was collected from a nursery that was found in Al Najaf City in September 2021, dried for 21 days at room temperature in the shade, and then ground to a powder for the extraction of phytochemical constituents.

Firstly, take the dry root of the *Cycas revoluta* plant and defat the root by the macerating method with n-hexane for two days. The powdered root material was subjected to Soxhlet extraction for 18 hours using a solvent mixture composed of 85% ethanol and 15% distilled water. After extraction, the solvent was evaporated under reduced pressure to obtain the crude extract. The resulting residue was suspended in 100 mL of distilled water and subsequently fractionated using a series of organic solvents of increasing polarity. Liquid-liquid partitioning was performed successively with chloroform (1:1 v/v, 100 mL), ethyl acetate (1:1 v/v, 100 mL), and n-butanol (1:1 v/v, 100 mL). Each fraction was collected separately. The n-butanol fraction was selected for evaluation of its potential anticancer activity.

### *2.2. A549 lung cell line*

A549 cells are human alveolar basal epithelial cells identified as adenocarcinomic. These A549 cells were established in 1972 by D.J.Giard and colleagues, who obtained and cultured lung tissue from an excised tumor of a fifty-eight-year-old Caucasian male [15]. The cells serve as models for investigating lung cancer and formulating drug therapies [16].

### *2.3. Chemicals and reagents*

1) Trypsin/EDTA from capricorn,Germany; 2) DMSO from Santacruz Biotechnology, United States; 3) RPMI 1640 from Capricorn, Germany; 4) MTT stain from Bio-World, USA; and 5) Serum Fetal bovine from Capricorn, Germany.

### *2.4. Cell culture maintenance*

A 10% fetal bovine serum, 100 units/mL of penicillin, and 100 $\mu$g/mL of streptomycin supplemented MEM was used for cell line culture. The cells were passaged with Trypsin EDTA and biweekly reseeded at a density of 50% confluence, and the cells were incubated at 37°C [17].

### *2.5. Cytotoxicity assessment using MTT assay*

The cytotoxic effects of *Cycas revolute* n-butanol root extract were evaluated using a 96-well plate MTT cell viability test. A549 cells, obtained from ATCC at passage 82 (±2 passages), were seeded with 1 × 10^4 cells from the cell lines [18]. Cells were treated with the tested substance (n-butanol extract) after one day or upon reaching a confluent-monolayer. After removing the media, twenty-eight $\mu$L of two mg/mL MTT solution was added (The MTT solution was prepared by dissolving 5mg of MTT in 1mL of 1 × PBS, followed by sterilization through filtration) and the cells were incubated for 90 minutes at 37 degrees Celsius to

assess cell viability following 72 hours of treatment. Removal of the MTT solution facilitated the solubilization of the leftover crystals in the wells using one hundred thirty $\mu$L of DMSO (Dimethyl-Sulphoxide), and then it was incubated for 15 minutes at 37 degrees Celsius with shaking [19]. By using a microplate reader at 492 nm, the absorbance was measured with the assay conducted in triplicate.

The cell growth Suppression rate, defined as [cytotoxicity percentage], was measured by using the equation:

$$\%\ \textbf{viability of cell}\ [\textbf{Treated cell absorbance}/\textbf{Non treated cell absorbance}]\ \times 100\%$$

$$\textbf{cytotoxicity} = 100 - \textbf{viability of cell}$$

The mycoplasma detection method was done by an enzymatic assay (MycoAlert) [20].

### 2.6. Statistical analysis

Using an unpaired t-test with GraphPad Prism 6, the data were statistically analyzed [21]. The values were presented as the mean ± SD of triplicate measurements, and the P value was considered significant if it was less than 0.05, which was considered significant [22].

## 3. Results and discussion

The present study shows that the n-butanol extract of *Cycas revoluta* roots exerts significant antiproliferative activity against A549 human lung adenocarcinoma cells, which is represented by the concentration-dependent cytotoxic activity demonstrated in the MTT assay as shown in Fig. 1. Furthermore, The $IC_{50}$ value was determined to be 4.564 $\mu$g/mL, indicating strong cytotoxic potential of the root extract against A549 cells (Fig. 2). Our findings contribute to the growing evidence of the anti-cancer potential associated with plant-based compounds, particularly in the context of lung cancer, which is an important leading cause of mortality due to cancer worldwide. These cytotoxic effects were further validated through morphological analysis of A549 cells using inverted microscopy, as treated cells exhibited signs of shrinkage, membrane blebbing, and loss of adhesion with respect to control untreated cells (Fig. 3). The observed bioactivity could be attributed to the broad spectrum of phytochemical constituents existing in *Cycas revoluta*, which previous investigations have reported the presence of several bioactive components, such as flavonoids, terpenoids, and non-protein amino acids. Although

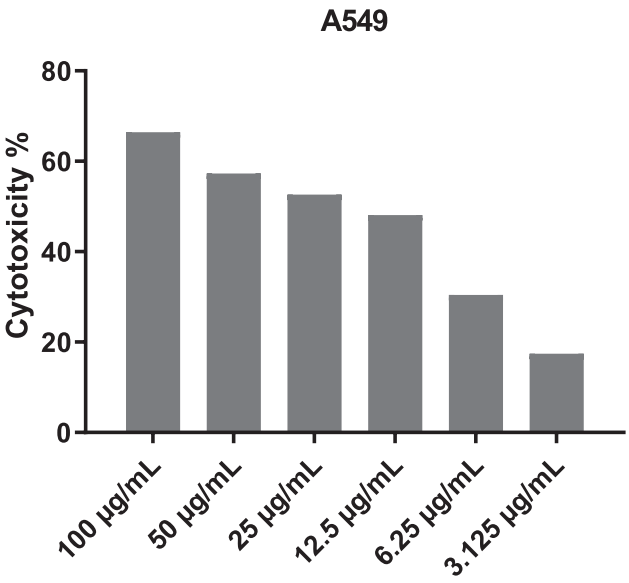



*Fig. 1. Cytotoxic effect of n-butanol root extract on A549 cell line (lung cancer).*

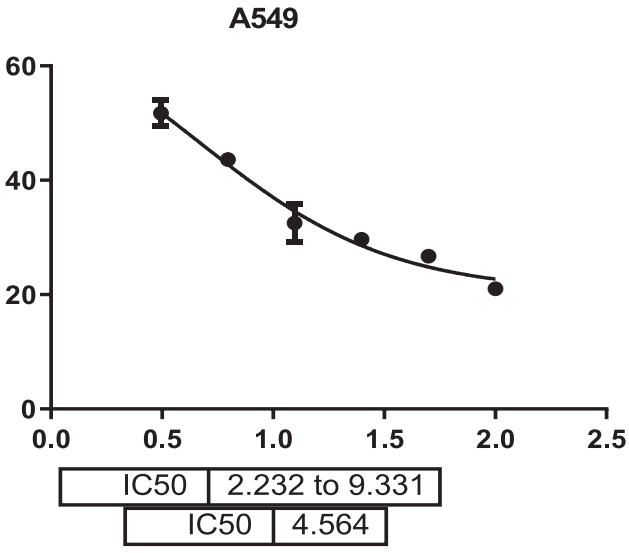
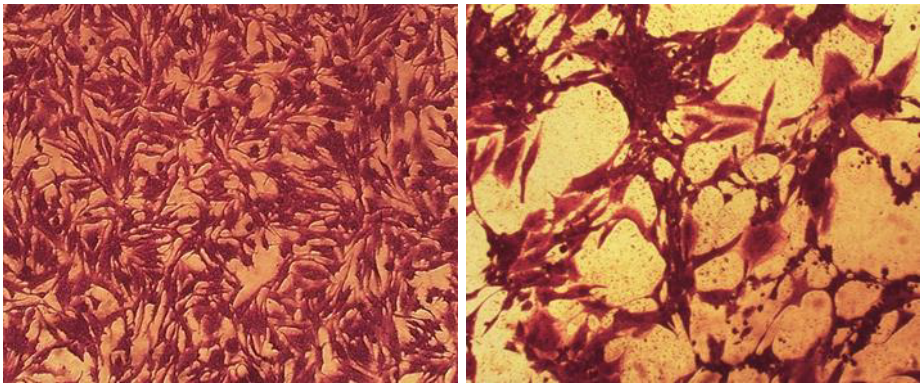



*Fig. 2. IC50 of n-butanol root extract on A549 cell lines.*

most phytochemical studies on *Cycas revoluta* focus on leaves and cones, comprehensive analyses show that the roots also contain diverse bioactive compounds including phenolic acids, saponins, and terpenoids (e.g., diterpenoids, triterpenoids). These phytochemical differences may partly explain the distinct biological activities of root extracts, and underscore the need for targeted compositional analyses in future work [13].

Such classes of compounds are well-reported for their anti-cancer activity, and they are known to act through several mechanisms, like induction of apoptosis, cell cycle arrest, and inhibition of angiogenesis



A B

*Fig. 3. Morphological picture of A549 cells in vitro, observed under a 10X inverted microscope using crystal violet stain. (A) Untreated A549 cells; (B) Treated A549 cells with n-butanol root extracts of Cycas revolute Thunb.*

[23, 24]. Although amentoflavone (a biflavonoid) found in *Cycas revoluta*—has been shown to induce G1 cell-cycle arrest and apoptosis in various cancer cell lines (e.g., non-small cell lung cancer) via pathways involving caspase-3/-8 activation and NF-$\kappa$B inhibition [25], the mechanism behind the cytotoxicity we observed in root extracts remains speculative. To clarify this, future studies should use targeted molecular and biochemical assays.

We don't use positive control, just negative control (which is the untreated cell line); because the aim of the study was only to evaluate the anticancer effect of the plant, not to make comparisons with other anticancer drugs.

The current results align with and extend previous reports of anti-cancer activity from different parts of *Cycas revoluta*, including reports of selective cytotoxicity against gastric cancer cells and protective effects in colon cancer models. However, this study represents the first demonstration of activity against lung adenocarcinoma cells specifically from the root-derived n-butanol fraction. This fraction was chosen based on its intermediate polarity, which would be expected to contain glycosylated flavonoids and other polar secondary metabolites that often demonstrate significant biological activity. The 72-hour treatment period used in our assay allowed for a comprehensive evaluation of both immediate cytotoxic effects and longer-term anti-proliferative consequences [9, 26].

These findings hold clinical significance when evaluated against the backdrop of existing challenges in lung cancer treatment. Targeted therapies and immunotherapies have enhanced outcomes for certain patients; however, there is a significant need for innovative therapeutic alternatives, especially for those who exhibit resistance to existing treatments. Plant-derived compounds have traditionally been vital sources of anti-cancer drugs, such as taxanes and vinca alkaloids. Current findings indicate that *C. revoluta* may be another potentially valuable source. Further research is necessary before clinical application, encompassing thorough toxicity studies, pharmacokinetic analysis, and assessment of potential synergistic effects with current chemotherapeutic agents [27–29].

The current study has several limitations that should be acknowledged. The in vitro nature of the experiments offers preliminary evidence of bioactivity that requires validation in more complex models. The reliance on a single cell line for initial screening restricts the generalizability across various molecular subtypes of lung cancer. The crude nature of the fraction suggests that the observed effects likely result from the combined activity of multiple compounds rather than a singular active principle. Future research must overcome these limitations by broadening testing to include additional cell lines and primary cultures, integrating three-dimensional culture models, and advancing to *in vivo* efficacy studies using suitable animal models.

The results offer promising initial evidence of anticancer potential, it's important to acknowledge that the experiments were limited to in vitro cell lines. To better understand the true therapeutic value of the root extract, future studies using animal models or clinical settings will be essential.

## 4. Conclusion

The n-butanol extract from the roots of *C. revoluta* exhibits notable anti-proliferative effects on A549 lung cancer cells, indicating its potential as a natural anti-cancer therapeutic agent. Additional research is required to identify the active components, clarify the molecular mechanisms involved, and assess the *in vivo* effectiveness. This research contributes to the

growing body of evidence suggesting that medicinal plants could be vital sources for developing novel anti-cancer therapies.

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## Ethical issue

None.

## Conflict of interest

The author confirms that there are no conflicts of interest related to this work.

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