Cytotoxic Potential of *Ficus palmata* Extracts on Lung Cancer Cell Lines (A549)

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Author’s contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

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ABSTRACT

Cancer accounts for 10 million deaths globally whereas the treatments available either have several side effects or non-effective due to multi-drug resistant property of cancer cells for long term use. Therefore, it is utmost important to find safe therapeutic drugs that have cytotoxic potentials against cancer cells. Medicinal plants are believed to have lesser side effects with huge therapeutic efficacy. One such species is *Ficus palmata* which is known to have several medicinal properties and great antioxidant potentials. Earlier it was reported that methanolic extract of leaves (FPLM) and aqueous extract stem bark (FPBA) of this plant have decent antioxidant properties. Therefore, the anticancer potentials of these plant extracts have been evaluated in addition of hydroxyl radical scavenging property. The results shown that FPBA represents higher antioxidant (IC$_{50}$ value= 242.46±11.26 µg/ml) and anticancer effects against lung cancer (A549) cell lines (IC$_{50}$ value= 69.74±2.12) whereas this plant extract have lower toxicity (IC$_{50}$ value= 249.61±7.31) on normal cell lines (3T3-L1) which indicate that FPBA can be a potential therapeutic option for cancer and oxidative stress. The study concluded that the stem bark aqueous extract having therapeutic properties against lung cancer. It is to be recommended that the bioactive compound responsible for its therapeutic properties need to explore against cancer cell by evaluating various other parameters such as apoptosis, cell cycle arrest, and toxic effect on cell morphology.

Keywords: *Ficus palmate*; lung cancer; antioxidant.
1. INTRODUCTION

Cancer is the major health concern which accounts for around more than 70% of deaths globally specially in middle- and low-income countries. Approximately 10 million deaths worldwide in 2020 was due to cancer, and the major cause of these deaths was specially lung cancer that accounts for 1.80 million deaths [1–3]. Lung cancer mortality rate is higher than the breast, colon and prostate cancer might be due to higher metastasis potential, and difficulties in diagnostic such as delayed in diagnosis [2,4]. The diagnosis of lung cancer is not easy because it cannot be easily seen or felt, and it’s also harder to realize by the patient until there are problematic symptoms exhibited, like chest pain, persistent cough, shortness of breath. According to a report the median time spent from the onset of symptoms to the initiation of treatment is around 138 days, this delay in diagnosis might be the hurdle for the early treatment of disease which can increase the mortality rate due to lung cancer [5]. Recently there are novel approaches reported for the detection and characterization of lung cancer based on artificial neural network and machine learning model which may be beneficial in prediction and detection of lung cancer [6,7]. Despite having several therapeutic interventions, and discoveries, still there are requirements of novel approaches for therapeutic potentials with lower side effects due to multi-drug resistance, several side effects and lack of selectivity [8–12]. Lung cancer mostly results from extensive use of smoking, tobacco exposure and decrease in physical activity which can increase the oxidative stress, atherosclerosis and induce the cancer [13]. To target these issues, there is a need to investigate for the better medicinal options to minimize the pathophysiological causes by reducing the oxidative stress and cure the lung cancer. Therefore, there is a requirement to investigate for the novel therapeutics from natural resources due their easy availability, economic, and lower side effects in prolonged use [14].

Several medicinal properties have been investigated for plant based natural products and metabolites for their potency to combat the oxidative stress, and its related disorder such as diabetes, gastrointestinal infection, microbial infection, DNA-damage, hyperlipidemia and neurodegenerative disease including cancer [15–31]. Medicinal plants such as Ficus species demonstrate better antioxidant potentials against cigarette smoke induce oxidative stress, and treat several diseases by inhibiting key regulatory enzymes during pathogenesis [16,20,21,23,30]. Moreover, it has been reported that fruits, leaves and stem-bark extracts of Ficus palmata have shown strong reducing property and enzyme inhibition potentials [23,32]. Hammoud & Shalaby [33] showed that leaves and fruits of F. palmata exhibited protection against diabetic induced liver and pancreas damage [34]. Several parts of F. palmata was also explored for their phytochemicals profiling, antimicrobial, nanoparticle’s preparation, and toxicological effects which indicates its potential role designing novel nutraceuticals and associated products [23,32,35]. Studies also confirmed the safety of F. palmata extracts that it does not affect the RAW 264.7 murine macrophages cell viability on up to 100 μg/mL [36].

Despite these several medicinal properties and safety of this plant, still there is very less information for its potential against cancer cells specially lung cancer. Therefore, it has been hypothesized that the leaves and stem bark extracts can be a potential therapeutic cure for lung cancer due to its high antioxidant nature and several medicinal properties. In this study the anticancer properties of leaf methanol extract and bark aqueous extract of F. palmata (FPLM and FPBA, respectively) have been explored by MTT assay for cytotoxic ability and hydroxyl radical scavenging assay has been performed to analyze the antioxidant potential.

2. MATERIALS AND METHODS

All the chemicals such as deoxyribose, ferric chloride, EDTA, citric acid, hydrogen peroxide, trichloroacetic acid, thiobarbituric acid, hydrochloric acid (HCL), methanol, Dulbecco’s modifications of eugal’s medium, DMSO, and MTT used in this study were of analytical grade and procured from Sigma-Aldrich (St. Louis, MO, USA).

2.1 Plant Material Preparation

Ficus palmata (Family: Moraceae) is a shrub or tree of up to 10-meter height and favorable to grow in cold region of desert area. The fresh leaves and stem bark of plant F. palmata were collected from mountain area of hail region, Qassim province, Saudi Arabia. Plant materials were rinsed with tap water and shed-dried at
room temperature for fifteen days after that it was grinded to make dry powder. The 10 grams of dry powder of plant leaves was extracted with 100ml of methanol (MeOH) solvent and 10 grams of dried stembark was extracted with 100ml of Aqueous solvent in Soxhlet apparatus for 12 hours. The extracts were evaporated using Rotatory evaporator and the remaining residue were stored at -20 °C for further analysis.

### 2.1.1 Hydroxyl radical quenching assay

The hydroxyl radical quenching effect of plant extracts was estimated according to the method of Halliwell et al. [37] with some modification [24]. Briefly, 3mM of deoxyribose, 0.1mM of ferric chloride, 0.1mM of EDTA, 0.1mM of citric acid and 4mM of hydrogen peroxide was prepared individually in 20 mM of phosphate buffer, pH 7.4. The 200μl of varying concentration of plant extracts were mixed with 200μl of each of the above prepared solvents and then this mixture was kept for incubation (1 hour) at 37 °C. Further the ice-cold trichloroacetic acid (0.2 ml, 15% w/v) and thiobarbituric acid (0.2 ml, 1% w/v in 0.25N HCL) was combined in reaction mixture and this mixture was kept in boiling water bath for thirty minutes. The mixture was refrigerated to cool for few minutes then its absorbance was measured at 532 nm. The percentage of hydroxyl radical scavenging potential was determined by using the formula given below and IC50 was calculated as described earlier:

\[
\%\text{ Hydroxyl radical scavenging activity} = \left( \frac{\Delta \text{Absorbance of control} - \Delta \text{Absorbance of test Sample}}{\Delta \text{Absorbance of control}} \right) \times 100
\]

### 2.2 Cytotoxicity Analysis

#### 2.2.1 Cell culture

Lung adenocarcinoma (A-549) and mouse embryonic fibroblast like pre-adipocytes (3T3-L1) cells cell line obtained from ATCC (Rockville, MD), were grown as monolayer in Dulbecco’s modifications of eugal's medium (DMEM) with L-glutamine & 4.5G/L glucose and further supplemented with 10% FBS (fetal bovine serum), penicillin/streptomycin (250 U/ml), gentamycin (100 μg/ml) and amphotericin B (1 mg/ml). The cell line was incubated at physiological temperature (37 °C) with 5% CO2 in humidified atmosphere to achieve growth confluency for 24 hours.

#### 2.2.2 MTT assay

To verify the cytotoxic impact of *F. palmata* extracts, cell viability experiment was performed with the conventional MTT-reduction assay with slight modifications [38]. Briefly, 3T3-L1 cells, and A-549 cell lines earlier seeded in a 96-well plate at the density of 5 × 103 Cells/well were taken and media was removed and replaced with varying concentrations of plant extract mixed in 0.5% DMSO and media (31, 62.5, 125, 250, 500 μg/ml) in triplicate and incubated for 48 hours. After 48 hours of incubation cell were treated with MTT (10 μl of 5 mg/ml) and incubated at 37 °C for another 4 h. Absorbance of the formazan product formation was read at 540 nm wavelength using VICTOR Nivo Multimode Microplate Reader, Perkin Elmer. The results were given as mean of three independent experiments. The percentage growth inhibition was calculated using the following formula mentioned below:

\[
\%\text{ Inhibition} = \left( \frac{\text{Absorbance of the control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100
\]

### 2.3 Statistical Analysis

All the samples were analyzed in triplicate and the results were expressed as mean ± S.D. IC50 value was calculated by Origin version 6.0 Professional software and the results were evaluated by using one-way analysis of variance (ANOVA) and two tailed Student’s t-test. Non-significant (ns), significantly different *P<0.05, **P<0.01 vs 0 μg/ml.

### 3. RESULTS

#### 3.1 Hydroxyl Radical Scavenging Effect

The results indicates that FPBA (F. palmata bark aqueous) extract (IC50 value= 242.46±11.26 μg/ml) showed significantly better hydroxyl radical scavenging potential than FPLM (F. palmata leaves methanolic) extract (IC50 value= 360.85±13.86 μg/ml), respectively in dose dependent manner (Table 1, Fig. 1).

#### 3.1.1 Cytotoxic potential of *F. palmata* extracts against 3T3-L1, and A549 cells

The toxicity potential of FPLM and FPBA extracts on lung cancer and normal cell lines, respectively were assessed, and reported in Fig 2 and 3, respectively. The plant extracts exhibited a dose-dependent inhibition on cell proliferation in both the cell lines after 48 hours of incubation. It has been noticed that the effect of plant extracts was more deleterious on lung cancer cell lines (A549) than the normal pre-adipocytes cell lines (3T3-
The IC₅₀ value of FPBA and FPLM extracts found to be significantly lower for A549 (IC₅₀ value= 69.74±2.12 and 255.35±9.86, respectively) than 3T3-L1 (IC₅₀ value= 249.61±7.31 and 544.48±17.42, respectively) cell lines (Table 1).

![Hydroxyl Radical Scavenging](image1)

**Fig. 1.** Concentration dependent hydroxyl radical scavenging effects of plant extracts (FPBA, FPLM); each value in the figure is represented as mean ± SD (n = 3)

![A549-Lung Cancer Cells](image2)

**Fig. 2.** Cytotoxic effect of *F. palmata* extracts on A549 cell lines at varying concentrations

![3T3 - Pre Adipocytes Cells](image3)

**Fig. 3.** Cytotoxic effect of *F. palmata* extracts on 3T3-L1 cell lines at varying concentrations

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Table 1. IC_{50} value of F. palmata extracts for antioxidant and anticancer properties.

|                      | A549-Lung Cancer | 3T3 - Pre adipocytes cell | Hydroxyl radical scavenging |
|----------------------|------------------|---------------------------|----------------------------|
| FPBA                 | 69.74±2.12**     | 249.61±7.31               | 242.46±11.2                |
| FPLM                 | 255.35±9.86**    | 544.48±17.42              | 360.85±13.86              |
| MANNITOL             | NA               | NA                        | 116.27±4.72               |

**values are significantly (P<0.01) different from 3T3 – L1 cell lines

4. DISCUSSION

Oxidative stress induced by excessive release of free radicals has been reported to stimulate cellular destruction and further leads to various diseases including cancer [11,12,39]. Since, secondary metabolites from natural products have been suggested for the quenching of free radicals and balance the redox process, therefore it is valuable to evaluate the antioxidant properties of plant extracts. Excessive production of hydroxyl radicals (•OH) are believed to causes macromolecules damage specially DNA which can ultimately results in various illness [40,41]. The medicinal plants having hydroxyl radical scavenging properties have direct role in their antioxidant potentials [42]. Our results are in agreement with previously published report [23], where it was established that F. palmata leaves methanolic extracts showed better antioxidant effects and have IC_{50} value for hydroxyl radical scavenging (≥ 250 µg/ml) which is nearly equivalent to the result of this study. Previously it has been reported that fruits of F. palmata showed antioxidant activity due their higher flavonoids content [32]. Alqasoumi et al. [35] reported that F. palmata aerial part was having germanal acetate, psoralene, bergapten, vanillic acid and psoralenoside which can be responsible for its reducing properties and other therapeutic potentials, moreover Iqbal et al. [23] found very similar compounds in stem bark aqueous extract of F. palmata which was believed to be responsible for their antioxidative properties.

It has also been noticed from these results that FPBA extract have significantly higher toxic against cancer cell lines and lower toxic against normal cell lines. This may be because of its higher antioxidant activity [23,34]. Our results are in correspondence with previous report that extracts possess strong antioxidant have higher cytotoxic activity against cancer cell lines [43].

Along with this current study several other studies reported the various medicinal aspects of F. palmata which suggests that this under-explored plant have strong therapeutic potential and can also be used as an anticancer drug [23,32,34–36]. The limitations of this study that the bioactive compounds screening, have not been performed, only one parameter for cytotoxicity (MTT assay) has been done, and the cytomorphological assay was not performed.

5. CONCLUSION

The work represented in this study confirms that FPBA have strong antioxidant potential and anticancer property against lung cancer (A549). There is an opportunity to explore the anticancer properties by touching various aspects such as apoptosis, cell cycle arrest, and pathways targeted by the bioactive compounds of FPBA extract.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Author has declared that no competing interests exist.
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