Journal of Cancer Research, Treatment and Prevention

Obafemi F A, et al., 2023- J Cancer Res Treat Prev
**Research Article**

# *Ficus benjamina* as a Candidate for Cancer Therapy Due to its Selective Toxicity Property

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Received Date: 05-17-2023

Accepted Date: 05-26-2023

Published Date: 06-03-2023



## Abstract

The study was conducted to examine the cytotoxicity of *Ficus benjamina* against Human Embryonic (HEK293T) cell lines. The leaves of *Ficus benjamina* were obtained and washed under running water, shade-dried to constant weight and ground into fine pounder, and extracted using chloroform. The chloroform extract was further separated into samples A, B, C, D, and E with the use of Thin Layer Chromatography (TLC). HEK293T cells were cultivated in DMEM or Dulbecco's Modified Eagle's Medium. The cells were then seeded at a density of 8 x 104 cells/mL in 96-well tissue culture plates, resulting in 4000 cells in 50 L of the medium in each well. With the use of the CellTitre-Glo 2.0 reagent, cytotoxicity was evaluated. In each well containing 50 liters of media, cells were treated by adding 50 liters of fresh media with a 2X concentration of the test sample and 2% DMSO, resulting in a final concentration of the test sample with 1% DMSO. After that, the treated cells were incubated for 48 hours at 37°C with 5% CO2. The luminescent signal generated was measured using a microplate luminometer with a 500 ms integration time per well.

The findings from the present investigation suggest that the suppression of human embryonic kidney (HEK293T) cells by Bortezomib was markedly greater ($p<0.05$) in comparison to the chloroform extracts derived from *Ficus benjamina* leaf. However, no noteworthy distinction was observed in the cases of extract instances A, B, C, D, and E ($p>0.05$). The extract of *Ficus benjamina* exhibited selective cytotoxicity towards HEK293T cells; having minimal impact on normal cells. The study also found that the extract had a more favorable therapeutic index than the anticancer drug Bortezomib. This makes *Ficus benjamina* a candidate for cancer therapy research.

**Keywords:** Cancer therapy; *Ficus benjamina*; Cytotoxicity; Cell line; Chloroform extract.

## Introduction

A category of illnesses known as cancer is characterized by the unchecked growth and division of aberrant cells. It is a major global health issue that has resulted in an overwhelming number of deaths worldwide [1]. In 2020, an estimated 19.3 million new cases of cancer and 10.0 million cancer-related deaths occurred globally. With an expected 2.3 million new cases, breast cancer in women was the malignancy with the highest rate of diagnosis, surpassing lung cancer. With an expected 1.8 million deaths, lung cancer continued to be the top reason for cancer-related mortality [2].

According to global cancer estimates, Asia had the highest number of cancer cases and deaths in 2020, accounting for 50% of all cases and 58.3% of cancer deaths. America had 20.9% of the cases and 14.2% of the deaths globally, while Europe had 22.8% of the cases and 19.6% of the deaths overall. Due to the varied distribution of cancer types and greater case fatality rates in these regions, the share of cancer deaths in Asia and Africa was larger than the share of incidence [2]. Due to demographic changes, rising risk factors brought on by globalization and economic expansion, and a bigger increase in transitioning nations than in transitioned countries, the worldwide cancer burden is projected to climb to almost 47% in 2040 [2].

The utilization of medicinal plants has been significant in the lives of people around the world. In rural communities, traditional medicine practitioners are generally recognized as the primary healthcare providers. The frequent use of traditional herbal medicine is often attributed to poor access to healthcare in Nigeria and inadequacy to manage healthcare costs.

According to the World Health Organisation [3], about half of the global population lacks access to healthcare and catastrophic health expenditures annually force about 2 billion people into poverty. Nigeria is currently experiencing a brain drain in the medical sector, resulting in a doctor-to-patient ratio of 1:9,083 [4]. To meet the WHO requirement of 23 doctors, nurses, and midwives per 10,000 population, Nigeria, with a population of over 200 million, will need at least 363,000 healthcare professionals [4]. Lack of financial risk protection makes purchasing health services a significant barrier to access in Nigeria. Out-of-pocket expenditures for healthcare are among the highest in peer countries, accounting for 71% of health spending (World Bank, 2022). Nearly half of Nigeria's citizens live below the poverty line, leading to a significant portion of the population either delaying seeking healthcare, resorting to unlicensed practitioners, or forgoing care entirely (World Bank, 2022) or resorting to traditional medicine practitioners. According to Mgbeahuruikea et al. [5], Africa is a continent that possesses an abundant supply of medicinal plants, and Nigeria is among the African countries that commonly incorporate complementary and alternative medicine alongside traditional beliefs to treat various illnesses [6].

The plant diversity in Nigeria provides a natural abundance of medicinal resources


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that are commonly employed for treating various types of illnesses. The utilization of complementary and alternative medicine has been prevalent among the local populace for generations and has been passed down through knowledge transmission to younger generations. There are several factors that have contributed to the growing popularity of traditional and herbal remedies in Nigeria, such as the unavailability of conventional treatment options, negative side effects associated with conventional therapy, and the cost of treatment [7].

In Nigeria, plant extracts are extensively utilized as significant sources of chemotherapeutic agents, despite the widespread use of synthetic drugs by most of the population. Medicinal plants have been employed for managing cancer for a considerable period, especially in developing countries like Nigeria [8].

Synthetic drugs used to treat cancer are frequently accompanied by harmful side effects. As a result, using readily available and cost-effective medicinal plants as an alternative can serve as a solution to the toxic side effects of synthetic drugs. Traditional medicine is frequently favored due to its affordability and accessibility, in contrast to biomedical treatments. This is especially true in Africa, where the population-to-traditional healer ratio can be up to 100 times higher than the population-to-medical doctor ratio [9].

The bioactive chemicals in medicinal plants are what cause their pharmacological effects. Clinical research and phytochemical testing have shown that herbal medicines have antitumor effects against different types of malignancies [8,10].

According to a study done in Ibadan, Nigeria, people were more likely to choose traditional medicine because they believed it to be more effective, accessible, and affordable [11]. Another cross-sectional study demonstrated that populations who believe in supernatural causes of disease exhibit an increased utilization of traditional medicine, and a corresponding decrease in the use of biomedicine [12]. The application of ethnobotanical properties of medicinal plants, known as ethnobotanical bioprospecting, is a significant approach to new drug discovery [13,14].

According to Gilman and Watson [15], *Ficus benjamina*, also known as the Weeping fig, is a tree that falls under the family Moraceae. It has been utilized for ornamental and hedging purposes for many years. *Ficus benjamina* is widely used as a houseplant due to its popularity. The plant is grown in various forms, such as natural-looking bushes, twisted or straight trunks, and interwoven trunks. The branches of the tree have a slight droop, giving it an elegant green appearance.

The Ficus genus includes a wide variety of plants that have a long history of being used medicinally to cure a variety of illnesses. Both traditional preventive medicines and therapeutic uses of these plants have been reported [16]. Numerous investigations into the therapeutic advantages of these plants have been done as a result of their potential medicinal properties.

One of the Ficus genus's most diversified species, *Ficus benjamina*, has been widely researched for its therapeutic potential [16,17]. This species has been utilized to cure


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a variety of illnesses because it has been discovered to have several medicinal characteristics. In traditional medicine, this plant's leaves, bark, roots, and fruits have been used for a variety of treatments, including the treatment of wounds, fever, and diarrhea [16]. The plant's extracts have also been demonstrated to have anti-inflammatory and antioxidant effects, making them a promising subject for further study in the creation of pharmaceuticals [17]. According to Gilman and Watson [15], *Ficus benjamina*, also known as the Weeping fig, is a tree that falls under the family Moraceae. It has been utilized for ornamental and hedging purposes for many years. Nowadays, *Ficus benjamina* is widely used as a houseplant due to its popularity. The plant is grown in various forms, such as natural-looking bushes, twisted or straight trunks, and interwoven trunks. The branches of the tree have a slight droop, giving it an elegant green appearance.

Anthocyanins, alkaloids, coumarins, carotenoids, flavonoids, glycosides, phenolics, polyphenols, saponins, tannins, triterpenoids, volatile components, and vitamins were all found in *Ficus benjamina* after a phytochemical screening on different parts of the plant [18]. Additionally, Batool et al. [19] and Naz et al. [20] have stated that *Ficus benjamina* has a distinct aromatic scent, which comes from the essential or volatile oil largely present in its green leaves. This volatile oil is mainly composed of alkaloids, saponins, flavonoids, and tannins.

According to Batool et al. [19] and Naz et al. [20], the seed oil of *Ficus benjamina* mainly contains naringenin, quercetin, and cinnamic acid lactose, while its organic product contains caffeic corrosive. The bark of the plant has stigma sterol, and its root bark contains Benjamin amide. Furthermore, according to Salem et al., [21] the *Ficus benjamina* leaf oil that is harvested throughout the day has a significant quantity of different chemicals. These substances include hydroxyl morphine, aspidospermine, nicodicodine, adenocarpine, lycocernuine, isoleucine, dasycarpidan, retronecine, and clemastine. They also include alpha-Pinene, abietadiene, cis-alpha-bisabolene, and gas.

According to Imran et al. [22], the stem's essential oil of *Ficus benjamina* contains 2-Pentanone, 9,12-Octadecadienoic acid, hexadecanoic acid, and palmitic acid. On the other hand, the root's essential oil has eight compounds, including arsenous acid, methanamine, cyclopentanone, methyl-2-phenylindole, cyclopropaneoctanal, 9,12-octadecadienoic acid, hexadecanoic acid, and palmitic acid.

The essential oils extracted from *Ficus benjamina* leaves contained a variety of compounds, according to Ogunwande et al. [23]. These include α-pinene (13.9%), abietadiene (9.7%), germacrene D-4-ol (8.4%), cis-α-bisabolene (8.2%), isobornyl acetate (5.0%), abietatriene (4.9%), sabinene (3.7%), and trace amounts of δ-3-carene, 3,7-dimethyl-2,6-octadienal, nonanal, safranal, carvone etc. According to Rahama and Mashi [24], *Ficus benjamina* contains various alkaloids, such as p-bromo atropine, crin amidine, solasodine, ibogaine, lutidine, cinchophen, and ajmaline, in its bark. Additionally, Benjaminamide, a new ceramide, has been identified from the plant's twigs. The medical benefits of *Ficus*


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*benjamina* include the treatment of conditions including whooping cough, whooping malaria, influenza, diarrhea, bronchitis, and heat seizures in children. The herb has historically been used to treat diseases like cancer, piles, leprosy, skin issues, inflammation, and malaria [22].

Imran et al. [22] conducted a study to evaluate the antimicrobial activity of different parts of *Ficus benjamina*, including the stem, roots, and leaves, against four bacterial and two fungal strains. The researchers found that the extracts and fractions from all parts of the plant showed significant antimicrobial activity, with the stem exhibiting the highest range of antimicrobial activity. The antimicrobial activity of the extracts and fractions was measured by the diameter of the inhibition zone (DIZ). The results showed that the butanol fractions exhibited the strongest antimicrobial activity, with the n-butanolic fraction of the stem showing the highest DIZ value of 22.83 mm against *B. subtilis*. Among the stem extracts and fractions, the methanol extract and n-butanolic fraction showed substantial activity against *P. aeruginosa* and *B. subtilis*, respectively. The n-hexane, chloroform, and ethyl acetate fractions displayed moderate antimicrobial activity, with the ethyl acetate fraction showing the highest DIZ value of 16.88 mm. In terms of antimicrobial potential against *B. cereus*, the stem extracts and fractions showed the following order of activity: methanolic > n-butanolic > ethyl acetate > chloroform > n-hexane. Overall, the results suggest that the stem extracts and fractions of *Ficus benjamina* possess potent antimicrobial activity against the tested microorganisms.

According to Imran et al. [22], the root extracts of *F. benjamina* had higher antimicrobial activity against fungal strains when the chloroform and ethyl acetate fractions were used, compared to the methanol and n-butanolic fractions. The diameters of inhibition zones (DIZ) of the root extract and its fractions of methanol, n-butanol, chloroform, ethyl acetate, and n-hexane were 14.60, 14.98, 17.15, 16.38, and 14.70 mm, respectively, against *A. niger*. A stronger antibacterial potential was also discovered in the butanol fraction from the leaves, with DIZ values of 19.50 mm and 19.75 mm against *B. cereus* and *C. albicans*, respectively. The leaf activity was moderate in methanol, n-hexane, chloroform, and ethyl acetate, with the chloroform fraction having the greatest value at 12 mm. The leaves n-butanol fraction, however, had significant efficacy against *B. cereus*. The antibacterial potential of the n-hexane and ethyl acetate fractions, on the other hand, was lower. The study's findings point to the possibility of using *F. benjamina* extracts as an antibacterial agent to prevent the growth of numerous harmful microorganisms.

## Methodology

### Collection and extraction of plant material

Fresh leaves of *F. benjamina* were collected from Ibadan, Oyo State, Nigeria. The leaves were identified by trained botanists and taxonomists. The leaves were rinsed under running water, shade-dried to constant weight, and ground to a fine powder for extraction. The ground powder was extracted using Chloroform as extraction solvent.


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Solvent extraction of *Ficus benjamina* was done using separatory funnels, according to the procedures of [25]. Thin Layer Chromatography (TLC) was then performed to get the different components of the chloroform extract according to the procedure outlined by Cai [26].

**Cell culture**

The cells (HEK293T) were grown in 150 $cm^2$ flasks of Dulbecco's Modified Eagle's Medium (DMEM) at 37°C with 5% CO2. Until cells were confluent, the media was changed every two days. After being subjected to trypsin-EDTA treatment, confluent cells were extracted. Then, cells were seeded at a density of 8 × 104 cells/mL in 96-well tissue culture plates, resulting in 4000 cells in 50 L of the medium in each well.

**Treatment with test samples**

To obtain the final concentration of the test sample with 1% DMSO, 50 L of fresh media containing 2X the final concentration of the test sample with 2% DMSO was added to the cell in 50 L of media already in each well. 48 hours were spent incubating the treated cells at 37 °C with 5% CO2. After 30 minutes of equilibration at room temperature, 100 LA CellTitre-Glo 2.0 reagent (Promega) was added. The content was then mixed by shaking for 2–3 minutes on a shaker before being incubated for 10 minutes at room temperature. A microplate luminometer (Tecan Infinite M1000 Pro) with a 500 ms integration time per well was used to measure the luminescent signal generated. The experiment was carried out three times. The final DMSO concentration in the culture medium (1%), on the other hand, was employed as the control. A positive control was used, consisting of bortexomib at 1 M.

The following formula was used to determine the percent inhibition or reduction in cell viability:

Inhibitory activity(%)=(Control group LO-Test Group LO)/(Control group LO) × 100

LO = Luminescence output [27].

Compounds showing ≥ 50% inhibition were further processed for IC50 determination.

**Cytotoxicity screening**

Using the CellTitre-Glo Assay, the test sample's *in vitro* cytotoxic activity was assessed [28,29].

**Statistical analysis**

The inhibitory potential was determined in triplicate; the mean of the triplicate value was determined using simple descriptive statistics. Statistical significance was determined using analysis of variance (ANOVA). The result was considered significant when $p<0.05$. Data was collected using Microsoft Excel and all analysis was performed using Statistical Package for Social Sciences (SPSS, IBM ver. 23).

**Results**

The results of the current study indicate that the inhibition of human embryonic kidney (HEK) cells by Bortezomib was significantly higher ($p<0.05$) when compared to the chloroform extracts of *Ficus benjamina* leaf. There was no significant difference in the extract instances A, B, C, D, and E ($p>0.05$) (Table 1).


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| Extract | A–E (10 µg/ML) | A–E (50 µg/ML) | BTZ (1 µM) |
|---|---|---|---|
| **A** | -42.74 ± 0.37[b] | -29.78 ± 0.05[b] | 68.27 ± 0.31[a] |
| **B** | -43.76 ± 0.43[b] | -42.65 ± 0.19[b] | |
| **C** | -25.34 ± 0.58[b] | -24.59 ± 0.40[b] | |
| **D** | -25.46 ± 0.83[b] | -31.03 ± 0.32[b] | |
| **E** | -23.46 ± 0.03[b] | -21.2 ± 0.44[b] | |

**Table 1:** Cytotoxic activity of *Ficus benjamina* chloroform extract on Human Embryonic Kidney cells. BTZ=Bortezomib; Samples A–E were extracts of *Ficus benjamina* leaf. Results are mean ± Standard Error of Mean of triplicate treatment.

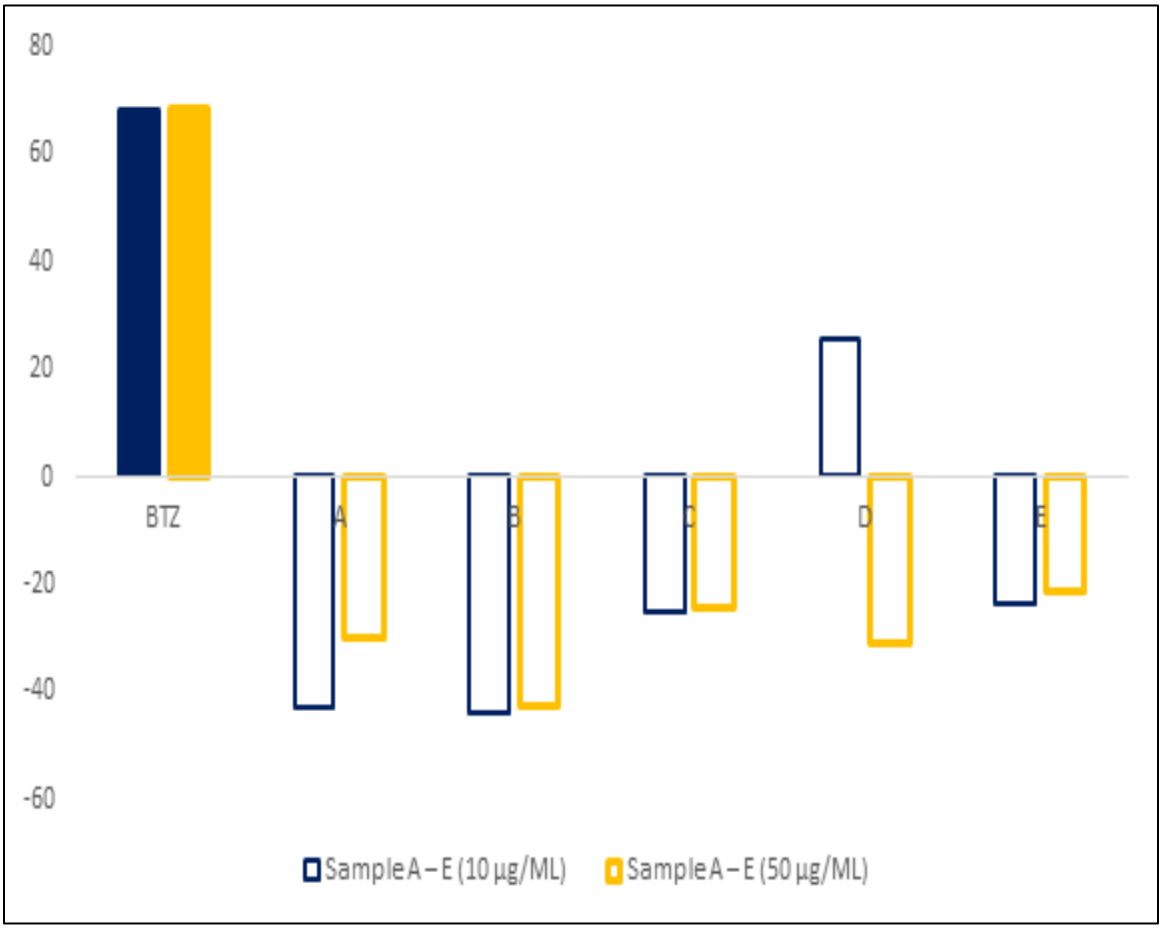


**Figure 1:** Cytotoxic activity of *Ficus benjamina* chloroform extract on Human Embryonic Kidney cells. BTZ =Bortezomib; Samples A–E were extracts of *Ficus benjamina* leaf. Results are mean ± Standard Error of Mean of triplicate treatment.


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## Discussion

The current study evaluated the cytotoxic effect of chloroform extract of *Ficus benjamina* leaves on normal human cells; the Human Embryonic Kidney cells (HEK).

The results of the current study indicate that the chloroform extract of *Ficus benjamina* leaf had no inhibitory effect on human embryonic kidney (HEK) cells. This suggests that the *Ficus benjamina* leaf extracts may have selective cytotoxicity, specifically targeting cancer cells while leaving normal cells unharmed. This selectivity is crucial for the development of cancer treatments as it reduces the risk of side effects and increases the safety and efficacy of the treatment. The Human embryonic kidney cell is a normal cell line [30], so samples not showing activity is an indication that they are not toxic to normal human cells [31]. A good candidate molecule against cancer cells should be selectively toxic to cancer cells; i.e. more toxic to cancer cells than to normal cells [32,33]. Sample C has a good selectivity index because it is significantly toxic to cancer cells and not to normal cells.

Additionally, when comparing the results of Bortezomib and *Ficus benjamina* leaf extract, the study found that Bortezomib, an anticancer drug, had lower selectivity than the *Ficus benjamina* leaf extract. The inhibition of human embryonic kidney (HEK) cells by Bortezomib was significantly higher ($p<0.05$) when compared to the chloroform extracts of *Ficus benjamina* leaf. This suggests that the use of *Ficus benjamina* leaf extract as a potential cancer treatment may have a more favorable therapeutic index, meaning the ratio of desired effects to undesired effects may be higher. This is particularly important for cancer treatment as it allows for a more targeted approach with minimal harm to normal cells.

This study suggests that the chloroform extract of *Ficus benjamina* leaf has potential for cancer treatment due to its selective cytotoxicity and favorable therapeutic index, although further research is needed to fully understand the mechanisms of action and potential for clinical use. It is important to note that the results of this study were obtained using cell lines and further studies are needed to confirm the results in vivo or in human models.

The *Ficus benjamina* leaf chloroform extract demonstrated diverse effects at different concentrations, however, statistical significance was not observed in its cytotoxic effect. Furthermore, the effects of the extract varied across different instances of extraction. These findings align with a previous study by Bhakat et al. [34], which reported varying effects of *Ficus benjamina* nanoparticles on Human Embryonic kidney cells (HEK293) at different concentrations.

In the present study, the cytotoxicity of the plant extract against HEK revealed negative values, indicating a low to minimal impact on healthy cells. In contrast, Bhakat et al. [34] reported that HEK293 treated with *Ficus benjamina* nanoparticle experienced cell death at concentrations of 6.25% and 3.125%. Moreover, the study observed morphological changes, cell wall damage, and cell size reduction within 6 hours at higher concentrations (50%, 25%, and 12.5%). At a


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lower concentration of 1%, cell wall damage was still visible, albeit to a lesser extent compared to higher concentrations.

The presence of rich phytochemicals in *Ficus benjamina* is suggested as the reason for the cytotoxicity results in this study [16]. These phytochemicals are responsible for the plant's potency and its potential to exhibit various medicinal properties [16]. The chloroform extract of *Ficus benjamina* leaves showed minimal toxicity toward HEK cells, which could be attributed to the plant's antioxidant potential. This effect is likely due to the combination of several antioxidant phytochemicals present in the extract [16]. Previous studies have reported that caffeic acid is one of the major antioxidant compounds present in the essential oil of *Ficus benjamina* leaves [22]. Caffeic acid possesses anticancer properties [35,36] and has demonstrated antitumor effects against several human cancers [37]. Additionally, it exhibits medicinal properties such as reducing oxidative stress and significantly inhibiting damage to DNA caused by free radicals [38]. Caffeic acid is also known to prevent cancer cell growth by inhibiting the HDM histone demethylase oncoprotein gene during cancer progression [39]. Therefore, the presence of caffeic acid in the *Ficus benjamina* extract could contribute to its negligible cytotoxic activity towards HEK cells.

## Conclusion

In conclusion, the study evaluated the cytotoxicity of the chloroform extract of *Ficus benjamina* leaves on normal human cells and found that the extract exhibited selective cytotoxicity towards HEK293T while having minimal impact on normal cells. The study also found that the extract had a more favorable therapeutic index than the anticancer drug Bortezomib. This makes *Ficus benjamina* a candidate for cancer therapy research.

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**Citation:** Obafemi FA, Umahi-Ottah G. *Ficus benjamina* as a Candidate for Cancer Therapy Due to its Selective Toxicity Property. J Cancer Res Treat Prev. 2023;1(3):148-158.
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