

Anti-proliferative effect of *Ficus pumila* Linn. on human leukemic cell lines

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ABSTRACT

Background: Cancer is one of the many diseases of global concern due to its high mortality rate with drug resistance becoming a major challenge to chemotherapy and this have propelled many cancer patients to seek alternative and complementary methods of treatment. The objective for this study was, therefore, to determine the antiproliferative activity as well as phytochemical, total phenolic content (TPC), and antioxidant activity of the stem and leaf extracts (FPS and FPL) of *Ficus pumila* (L.) using standard methods.

Methods: The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was used to evaluate anti-proliferative effect and spectrophotometric-based assays for antioxidant and TPC. Phytochemical constituents were accessed by standard methods.

Results: The hydroethanolic extracts of the leaves and stems were rich in tannins, general glycosides, saponins, terpenoids, alkaloids, flavonoids (leaves only), and sterols (stem only). Strong total antioxidant activities were observed with FPL and FPS with EC₅₀ values of 0.07 mg/ml and 0.089 mg/ml, respectively. All the crude extracts showed anti-proliferative effect towards the three human leukemic cell lines used (Jurkat, CEM, and HL-60). However, FPL gave the strongest inhibition concentration at 50% values of 130.97 µg/ml (Jurkat) and 56.31 µg/ml (HL-60).

Conclusion: These findings suggest that crude extracts of FPS and FPL have anti-proliferative effect on the leukemia cells. The antioxidant properties of the plant including phenolics may be partly responsible for the anti-proliferative activity. Further studies are required to isolate chemical components of the plant and establish their anti-proliferative activities and mechanism of action.

Keywords: Anti-proliferative, Antioxidant, *Ficus pumila*, Phenolics, Phytochemical

INTRODUCTION

Cancer is one of the most severe health problems in both developing and developed countries worldwide. Among the most common types of cancers are lung, stomach, colorectal, liver, and breast. Lung cancer is the most commonly

diagnosed cancer in men whereas breast cancer is the most common type in women.¹ Curative surgery is the first option for patients with early-stage cancer while radiotherapy and chemotherapy have proven to be effective treatments for patients in the advanced stages. However, the curative effect of traditional chemotherapeutic drugs is limited and their

side effects, such as neurological and/or renal and cardiac toxicity, are serious.²

Ficus pumila, synonymous to *Ficus repens*, is an ornamental plant from the family Moraceae. It is native to East Asia, specifically South China through to Malaysia.³ Ornamental plants, such as *F. pumila* are grown for only their aesthetic value. According to Yong et al.,⁴ the leaves of this plant have antimicrobial, antileishmanial, and anti-inflammatory effects among many other medicinal benefits. These benefits could be attributed to the wide array of chemicals contained in it. This plant, like all other ornamental plants, is very likely to contain certain important phytochemicals with pharmacological properties that could be useful.³

The objective of the current study was to assess the anti-proliferative effect of 50% hydroethanolic extracts of *F. pumila* leaves and stem, phytochemical and total phenolic content (TPC) and antioxidant activity.

METHODS

Cell lines and reagents

The cell lines used (CEM, Jurkat, HL-60 and PNT2) were obtained from RIKEN BioResource Centre Cell Bank (Japan). Culture media (RPMI and α -MEM), 96 well plates, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye, isopropanol, HCl, trypan blue solution, absolute ethanol, foetal bovine serum (FBS), antibiotics (penicillin and streptomycin), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and phosphate buffer saline were obtained from Sigma-Aldrich Company (St. Louis, MO, USA).

Plant and preparation

The leaves and stems of *F. pumila* were collected in November, 2013 (before 9 am) from the lawns of the Republic Hall of KNUST Kumasi campus. The plant was certified at the Department of Herbal Medicine (KNUST, Kumasi), and a specimen was deposited at the department's herbarium (voucher number KNUST/HM1/2014/L093). The different parts of the plant were then washed, air-dried, pulverized and packaged in zip-locks for further use. Preparation of 50% hydroethanolic extraction of the plant leaves and stems were carried out separately, by suspending 50 g of the powder of each part in 500 ml of 50% ethanol (50:50 v/v). The extraction was done by cold maceration for 24 hrs at room temperature on a shaker. The extracts were filtered through cotton wool, concentrated using a rotary evaporator⁵ and freeze-dried to obtain the *F. pumila* hydroethanolic leaf (FPL) and stem (FPS) extract.

Phytochemical screening

Qualitative analysis for phytochemicals was carried out to determine the presence of phytochemicals in both the

powdered samples and extracts of *F. pumila* using standard methods.⁶⁻⁸ Phytochemicals tested were tannins, saponins, general glycosides, alkaloids, flavonoids, triterpenes, and sterols.

Determination of total phenols

Total phenolic content (TPC) of FPL and FPS was determined using the Folin–Ciocalteu assay⁹ with slight modification. To a volume of 10 μ l of sample, 790 μ l of distilled water was added. The concentration of the FPL and FPS extracts tested was 5 mg/ml. 50 μ l of Folin–Ciocalteu reagent was added to the diluted samples and thoroughly mixed. The mixtures were incubated in the dark for 8 mins. Subsequently, 150 μ l of 7% Na₂CO₃ was added before incubation of the mixture for 2 hrs in the dark at room temperature. Triplicate experiments were performed. The absorbance was read at a wavelength of 750 nm using a microplate reader (Tecan Infinite M200, Austria). Gallic acid (GA) was used as the standard phenolic compound. A GA calibration curve was plotted and used to determine the phenolic content. The results were expressed in milligrams of GA equivalents per 100 g dry mass (mg GAE/100 g DM).

Antioxidant assay

The antioxidant activity of FPL and FPS extracts was determined using the free radical scavenging activity by DPPH method with some modification.¹⁰ Methanolic solution of DPPH (0.5 mM) was added to equal volumes of various concentrations of each extract (concentration range 0-5 mg/ml). After 20 mins incubation at room temperature, the absorbance was read at a wavelength of 517 nm (Tecan Infinite M200 Pro plate reader, Austria). The inhibition concentration at 50% (IC₅₀) value of each extract was calculated from the following formula:

$$\% \text{ Antioxidant activity} = [(A_0 - A_1) / A_0 \times 100]$$

Where A₀ is the absorbance of negative control (methanol), and A₁ is the absorbance of test sample with DPPH. Butylated hydroxytoluene (BHT) was used as standard control. Triplicate experiments were performed. The EC₅₀ value, which is the concentration of the extracts that can cause 50% free radical scavenging activity was determined.

MTT assay

L-RPMI and α -MEM culture media, respectively, supplemented with 10% FBS, containing penicillin, streptomycin, and L-glutamine were maintained in culture at 37°C in the presence of humidified 5% CO₂ atmosphere. The tetrazolium-based colorimetric assay (MTT) was used to determine the cytotoxicity of *F. pumila* on the cancer and normal cell lines.¹¹ Cells were seeded into the 96-well plates at the concentration of 1 \times 10⁴ cells/well, treated with varying concentrations of the plant extracts (0-1250 μ m/ml and incubated as indicated above for 72 hrs. A color control

plate was also setup for each extract including the positive control, curcumin. MTT solution (0.5 mg/ml) was added to each well on the plate, and incubation continued for further 4 hrs. The reaction was stopped with acidified isopropanol solution, and the plate incubated in the darkness overnight at room temperature before reading the absorbance at 570 nm using a microplate reader (Tecan Infinite M200 Pro, Austria). The percentage cell viability was determined as follows:

$$\% \text{ Cell viability} = \frac{[A_0 - A_1]}{A_0 - A} \times 100\%$$

Where A_0 = Mean absorbance of control wells

A_1 = Mean absorbance in test wells

A = Mean absorbance of blank wells

Percent the IC_{50} values were determined from the plot of percent cell viability on the y-axis against extract concentrations on the x-axis.

Statistical analysis

Data were analyzed using GraphPad Prism 5 for Windows and Microsoft Excel 2010. The experimental results were expressed as the mean \pm standard error of the mean. Data were assessed by one-way ANOVA followed by Newman-Keuls multiple comparison test. Values for which $p < 0.05$ was considered as statistically significant.

RESULTS

Phytochemical constituents

Table 1 shows the phytochemical constituent of the powdered raw sample and hydroethanolic extract. The solvent system was effective in extracting majority of the phytochemical contents of raw samples. The extracts contained tannins, saponins, general glycosides, alkaloids, flavonoids, triterpenes, and sterols.

Table 1: Phytochemical content of powdered samples and hydro ethanolic extracts.

Phytochemical	Leaves		Stem	
	Powdered sample	FPL	Powdered sample	FPS
Tannins	+	+	+	+
Saponins	+	+	+	+
General glycosides	+	+	+	+
Alkaloids	+	+	+	+
Flavonoids	+	+	-	-
Triterpenes	+	+	+	+
Sterols	-	-	+	+

-: Absent, +: Present, FPS: *Ficus pumila* stem extract, FPL: *Ficus pumila* leaf extract

TPC

The TPC of extracts, as assessed against GA standard is as shown in Figure 1. A linear plot of GA standard produced a straight line ($y=0.029x + 0.0657$, $R^2=0.9978$). The TPC of 5 g each of FPS was significantly higher ($p < 0.001$) compared with an equal amount of FPL.

Antioxidant activity

The antioxidant activity of FPS, FPL and standard BHT is shown in Figure 2. FPS and FPL showed a dose-dependent increase in activity similar to that of standard, BHT. Both FPS and FPL showed strong antioxidant activities which were similar.

Anti-proliferative activity of curcumin and extracts

The activity of extracts and the standard, curcumin on the Jurkat cells are as shown in Figure 3. FPL had stronger inhibitory activity against the cells with IC_{50} value of 130.97 $\mu\text{g/ml}$ compared to FPS. A concentration-dependent effect was observed for both extracts as was observed for curcumin. The effect of the extracts on human promyelocytic leukemic cells (HL-60) is shown in Figure 4. Similarly, FPL showed a stronger inhibitory activity with IC_{50} value of 56.31 $\mu\text{g/ml}$ compared to FPS. The anti-proliferative effect of the extracts and standard curcumin on human T-lymphoblastoid leukemia (CEM) cells is shown in Figure 5. Both FPL and FPS showed weak inhibitory activity towards the proliferation of CEM cells ($IC_{50} > 1000 \mu\text{g/ml}$).

The anti-proliferative effect of curcumin and extracts on normal human prostate cells (PNT2) are shown in Figure 6. Curcumin inhibited the growth of normal cells ($IC_{50}=15.01 \mu\text{M}$) whereas hydroethanolic extracts of *F. pumila* had no significant inhibitory effect.

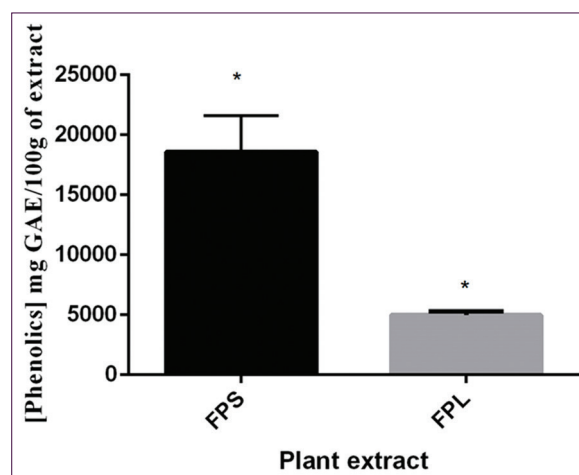


Figure 1: Total phenolic content of *Ficus pumila* stem and *F. pumila* leaves extracts. Each column represents a mean \pm standard error mean. * $p < 0.001$.

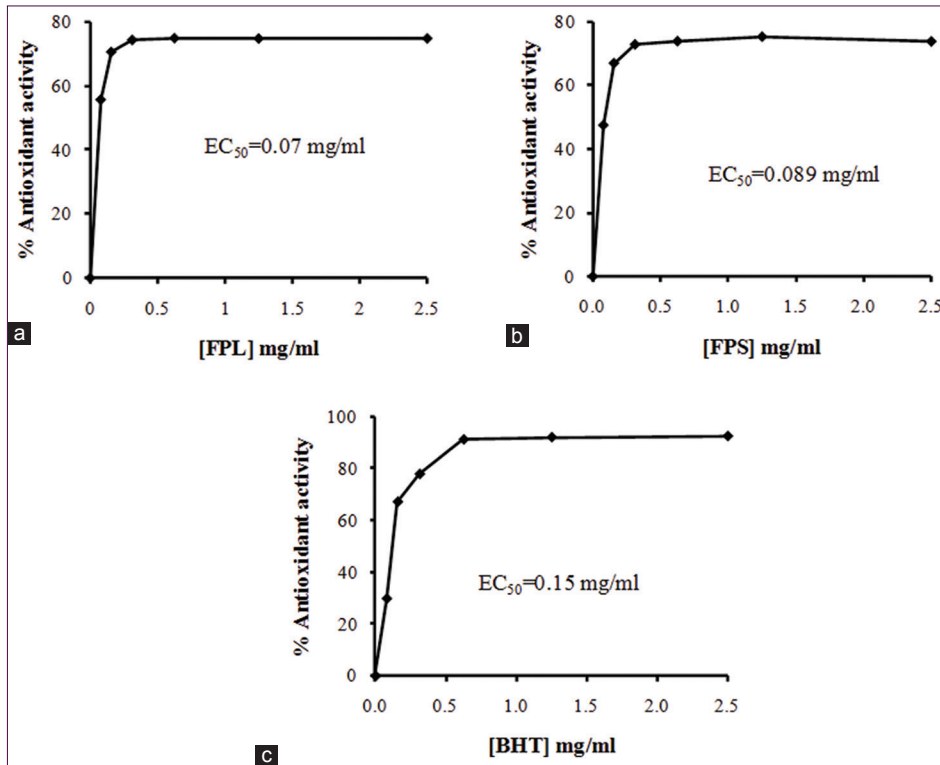


Figure 2: Antioxidant activity of (a) *Ficus pumila* leaves, (b) *F. pumila* stem, and (c) standard, butylated hydroxytoluene. Each point represents a mean of three determinations.

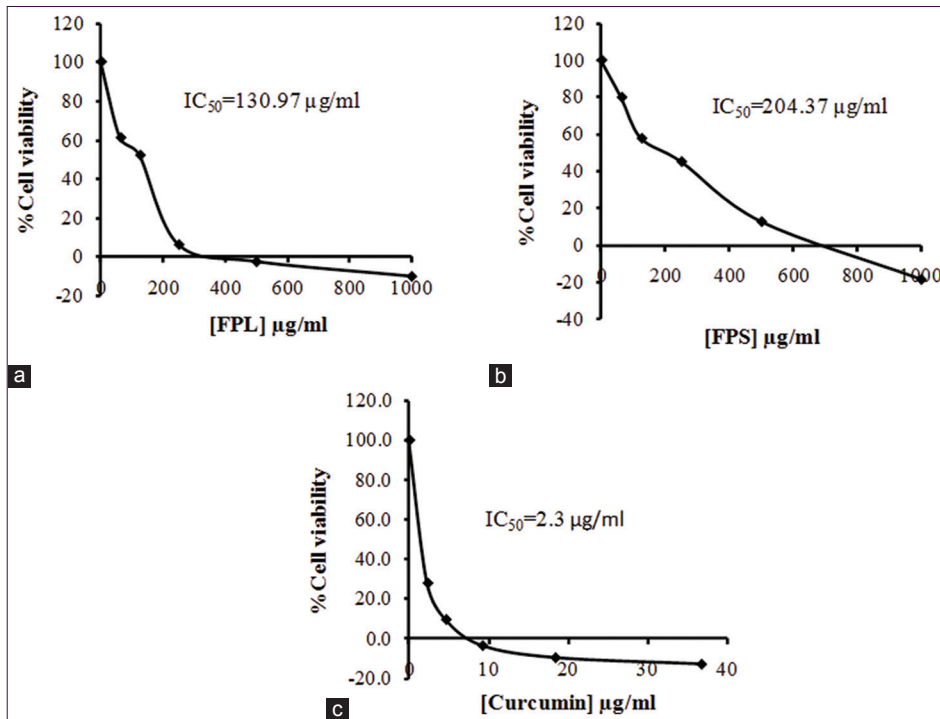


Figure 3: Anti-proliferative activity of (a) *Ficus pumila* leaves, (b) *F. pumila* stem, and (c) curcumin, on Jurkat cell. Each point represents a mean of three determinations.

DISCUSSION

Novel therapies are needed to overcome the challenges to cancer therapy. In the present study, we investigated the anti-leukemic cancer activity of the hydroethanolic extracts

of the leaves and stems of *F. pumila*. A wide range of phytochemicals which include tannins, saponins, glycosides, alkaloids, flavonoids, triterpenes, and sterols were detected in both leaf and stem extracts. However, sterols and flavonoids were absent from the leaves and stems, respectively. The

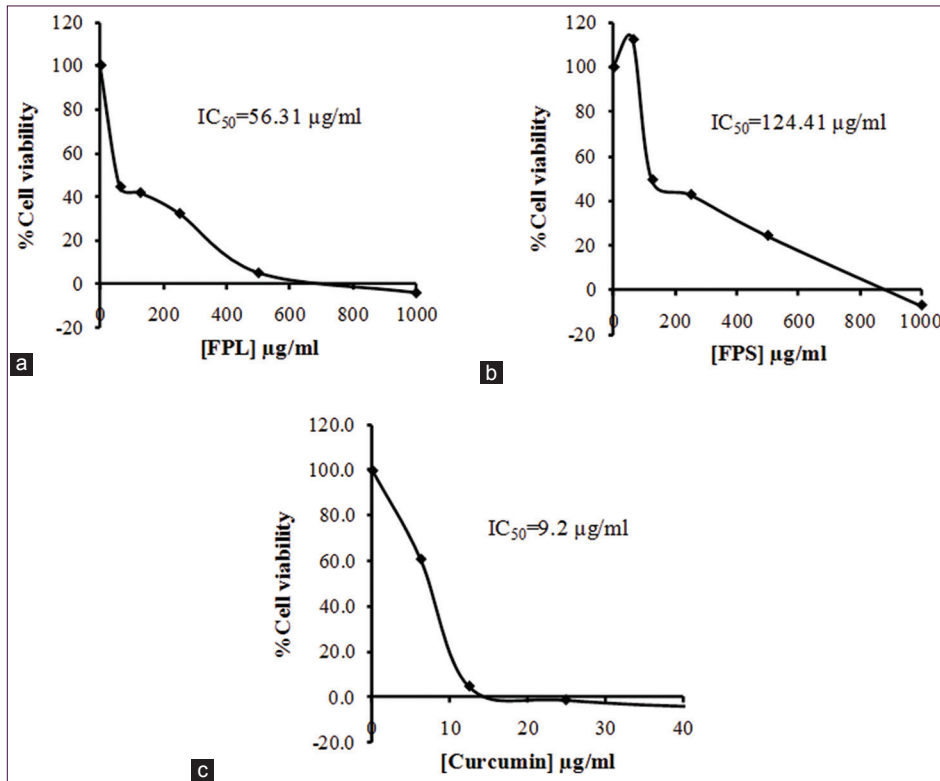


Figure 4: Anti-proliferative activity of (a) *Ficus pumila* leaves, (b) *F. pumila* stem, and (c) standard, curcumin on HL-60 cell line. Each point represents a mean of three determinations.

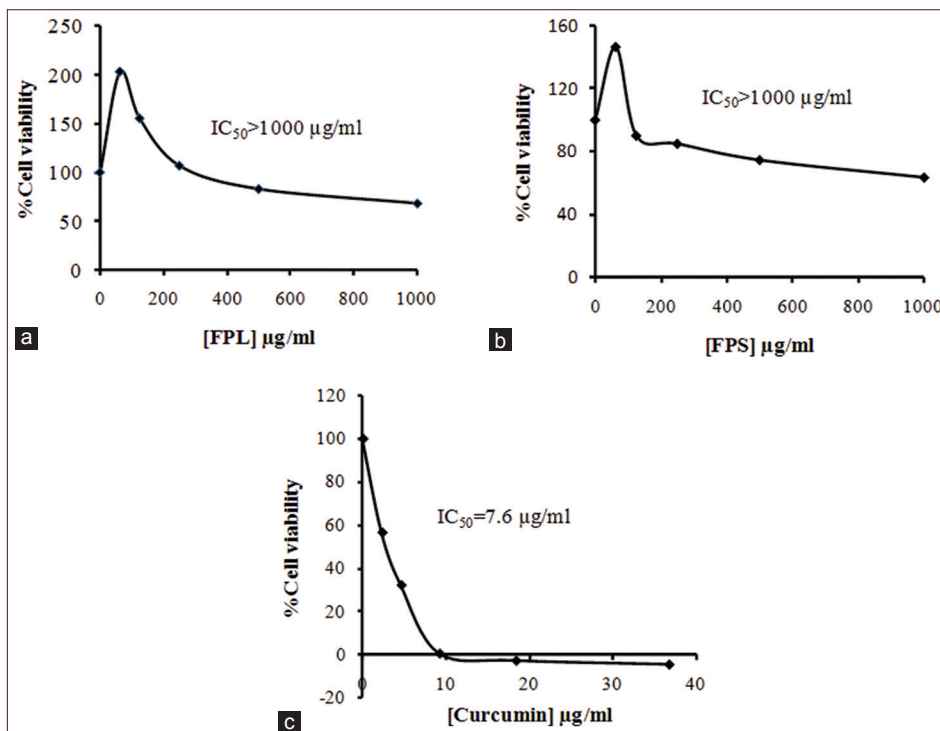


Figure 5: Anti-proliferative activity of (a) *Ficus pumila* leaves, (b) *F. pumila* stem, and (c) curcumin on CEM cell line. Each point represents a mean of three determinations.

phytochemicals present in the plants powdered sample (without extraction) were the similar to those hydroethanolic extracts. Thus, it appears 50% ethanolic solution was an appropriate solvent for the extraction process.

The phenolic content of the plant was very significant ($p < 0.001$) especially in the stem. This is comparable to previous work done,¹² which revealed that the TPC in acetone extract of *Ficus capreifolia* and *Ficus coralata* were 4.73

and 8.23 mg GAE/g dry weight, respectively. The differences in the TPC could be due to the type of solvent used in the extraction and the specie variety.

From Figure 2, it was observed that the mean % antioxidant activity increased as concentration increase. The EC_{50} values of 0.07 and 0.089 mg/ml for FPL and FPS respectively, suggest that the crude extracts possess strong free radical scavenging activity. Work done has shown that *Ficus* species (*Ficus virosa* and *Ficus ingens*) had EC_{50} values of 0.03 mg/ml and >2.5 mg/ml, respectively.¹³ This confirms that the *F. pumila* may have comparatively higher antioxidant activity (EC_{50} value of 0.07 mg/ml for the leaves and 0.089 mg/ml for the stem) compared to the other species. *Ficus* species are a rich source of polyphenolic compounds, which are responsible for strong antioxidant properties that help in prevention and therapy of various oxidative stress related diseases including cancers.¹⁴ Thus, the phenolic content of the crude extracts measured in this study may be partly attributed to the antioxidant properties of the extracts. Accordingly,¹⁵ phenolic compounds increase plasma antioxidant capacity, which is required in cancer chemotherapy. Antioxidants play a very relevant role in the control of many health disorders relating to oxidative stress and free radical activities of which cancer is major outcome.¹⁶

From the MTT data, it was observed that all the crude extracts had anti-proliferative activity against the three human leukemic cancer cell lines (Jurkat, CEM and HL-60), which was concentration-dependent. The HL-60 cell line was more sensitive to the anti-proliferative activity of the

F. pumila extracts. The FPL was more cytotoxic exhibiting three-fold stronger anti-proliferative activity towards the cell line than the stems (FPS). Similarly, the leaves showed stronger anti-proliferative activity towards Jurkat cells than the stem cells. Both crude extracts showed rather weak anti-proliferative activity against CEM cell line compared to the other cell lines. This can be attributed to the fact that CEM cell line is a multidrug-resistant leukemia cell line,¹⁷ therefore, the cells were resisting the inhibitory action of the extracts. Interestingly the extracts showed strong antioxidant properties. The free radical scavenging activity is crucial in cancer therapy since reactive oxygen species are closely involved with various pathological events such as cancers, aging and, inflammation among others.

Similar cytotoxic effects have been observed in previous work done.¹⁸ Their study revealed that crude extracts of *F. pumila* and *Flemingia strobilifera* were cytotoxic (IC_{50} values of 131 and 81 μ g/ml respectively) against MT-4 human leukemia cancer cell lines which is comparable to that observed for FPL on JURKAT and HL-60 cell lines. However, in the same study, better anti-proliferative effect was achieved for *F. pumila* with chloroform extraction (IC_{50} value of 23 μ g/ml). This is suggestive of the fact that different solvents may affect the concentration of anticancer agents in the extracts differently.

It was however, observed that the extracts especially the leaf extracts had no significant anti-proliferative effect on the normal prostate cell line in that in the presence of the extract and even at higher concentrations, the normal prostate

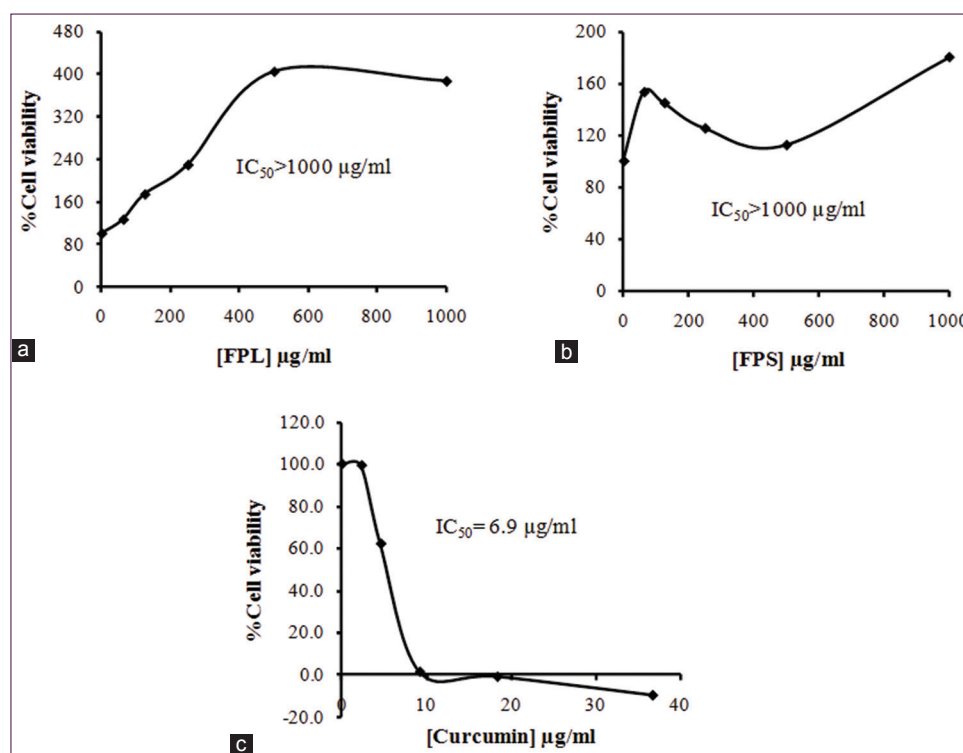


Figure 6: Anti-proliferative activity of (a) *Ficus pumila* leaves, (b) *F. pumila* stem, and (c) standard curcumin on normal prostate cell line (PNT2). Each point represents a mean of three determinations.

cell lines were still viable. In the case of the stem extracts, the cell viability increased at higher extract concentrations which were the exact opposite in the case of the standard drug curcumin. The selectivity indices of both leaf and stem extracts in Jurkat and HL-60 cell lines was in the range 4.9-17, indicating rather a good selectivity of the extracts for the human leukemia cell lines compared to normal human cells.

CONCLUSION

This study has shown that hydroethanolic extracts of *F. pumila* have selective anti-leukemic activity towards two human leukemia cell line and thus contains constituents with potential as anti-leukemic agent in humans. *F. pumila* is also a rich source of phenols and antioxidants, and these may partly account for the anti-leukemic activity of the extracts. Besides its anti-proliferative effect on leukemic cells, the plant has minimal cytotoxic effect on normal cells which most cancer drugs lack. Bioassay-guided fractionation of extracts and isolation of pure compounds are warranted to identify the anticancer principle of *F. pumila*.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee.

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