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**Abstract**

**BACKGROUND:**

Flueggea leucopyrus Willd is a shrub grown in many parts of the dry zones in Sri Lanka. The leaves of F. leucopyrus has been used for treating cancer in the traditional system of medicine in Sri Lanka. Hence, this study was performed to analyze the antioxidant and antiproliferative properties of the aqueous extract of the leaves of F. leucopyrus on HEp-2 cells.

**METHOD:**

The aqueous extract of F. leucopyrus leaves (AEFLL) was freeze dried. Total phenolic content was assayed using Folin Ciocalteu reagent. Antioxidant activities of the extracts were evaluated using in vitro assays: inhibition of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging and 2-deoxy-D-ribose degradation assay. Nitric oxide radical scavenging activity was determined by using Griess reagent. The MTT, LDH assays and protein synthesis were used to study antiproliferative and cytotoxic activities against the Hep-2 cell after 24 hour exposure. DNA fragmentation and microscopic examination of cells stained with a mixture of ethidium bromide/acridine orange were used to visualize apoptosis in HEp-2 cells treated with the AEFLL.

**RESULTS:**

The total phenolic content of the extract was 22.15 ± 1.65 (w/w) % of gallic acid equivalent. The values for EC50 were 11.16 ± 0.37, 4.82 ± 1.82 and 23.77 ± 3.16 μg/mL for DPPH radical scavenging, nitric oxide radical scavenging activity and 2-deoxy-D-ribose degradation assay respectively. The EC50 with MTT and LDH assays were 506.8 ± 63.16 and 254.52 ± 42.92 μg/mL respectively. A dose dependent decrease in protein synthesis in HEp-2 cells was shown with an EC50 value of 305.84 ± 12.40 μg/mL. DNA fragmentation and ethidium bromide/acridine assays showed that the AEFLL induces apoptosis in HEp-2 cells. These results were in conformity with the morphological changes observed in the cells treated with the AEFLL. The brine shrimp bioassay showed that the AEFLL had no lethality over the concentration range of 50-500 μg/mL.

**CONCLUSIONS:**

Aqueous extract of the leaves of F. leucopyrus extract demonstrated antioxidant activity in vitro. Further it showed antiproliferative properties and induced apoptosis in HEp-2 cells.

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Figure 1

**The percentage cytotoxicity on** ***HEp-2*** **cell line as determined by MTT assay, after 24 hour treatment with the AEFLL.** The data are presented as mean ± SD of three independent experiments. The linear segment of the dose response curve was used to determine EC50 value.

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Figure 2

**The percentage LDH released after 24 hour treatment with the AEFLL on** ***HEp-2*** **cell line.** The data are presented as mean ± SD of four independent experiments. The linear segment of the dose response curve was used to determine EC50 value.

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Figure 3

**Light micrographs of** ***HEp-2*** **cell line after 24 hours of incubation with the AEFL at different concentrations.** Cells treated **A**-negative Control; **B**-Positive Control (Camptothecin); **C**- 100 μg/mL; **D** - 300 μg/mL. Live cells have definite morphology and dead cell are rounded. Reduction in cell density also observed in positive control and the AEFL treated cells. Acridine orange-ethidium bromide (AO/EB) fluorescent staining detection of apoptotic morphology in *Hep2* cells treated with the AEFL at different concentrations are depicted in the bottom row. **E**-negative control; **F**- Camptothecin as the positive control (5 mM; 25 μL); **G**- 400 μg/mL, **H** - 800 μg/mL. This figure represents the results of at least 3 independent experiments. Green arrows: live cells, greenish yellow: apoptotic cells (some cells are fragmented and faded color), orange red: late apoptotic cells. (Original magnification 40×).

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Figure 4

**Agarose gel electrophoresis shows DNA fragmentation indicating induction of apoptosis by the AEFLL in** ***HEp 2*** **cells.** Lane 1: treated with 200 μg/mL, lane 2: DNA molecular weight marker.

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