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Comparative Cytotoxic Activities of the Flavonoid-Rich Ethyl Acetate Fruit Extract of *Pouteriacampechiana*baehni (Sapotaceae) in K562 Leukemic Cancer Cell Lines and Healthy Human Whole Blood Cells

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Abstract: *The fruit of Pouteriacampechianahas been previously reported to contain flavonoids and polyphenolic substances with high in vitro anti-oxidative effects. Its anti-tumorigenic activities have not been previously demonstrated. This study seeks to demonstrate the cytotoxic effects of the flavonoid-rich ethyl acetate fraction of the fruit extract of P. campechiana(EAFFPC) against K562 leukemic cell lines and healthy human whole blood cells. The standardized MTT-dye cell viability assay was carried out to measure the cytotoxicity of EAFFPC against the aforementioned cell lines cultured in RPMI. The assay showed that at a 60 µg well concentration, EAFFPC exhibited a 55.4% cell viability which is significantly lower than the cell viability obtained with 10 µg of vincristine. In contrast, EAFFPC demonstrated concentration-dependent cytoprotective properties in healthy human whole blood cells (HHWBC). This study confirms specific non-concentration-dependent cytotoxic effects on K562 leukemic cell lines while exhibiting a concentration-dependent cytoprotective effects in HHWBC.*

Keywords: *Pouteriacampechiana, K562 leukemic cell lines, cytotoxic, healthy human whole blood cells*

I. Introduction

About 60% of the volume occupied by whole human blood, known as the plasma, consists of hormones, antibodies, enzymes, glucose, fats and salts while the rest of the 40% consists of erythrocytes, leukocytes and *platelets*, In leukemia, the bone marrow and other blood-forming organs produce increased numbers of immature or abnormal leukocytes. These suppress the production of normal blood cells, leading to anemia and other symptoms. Symptoms include bleeding and bruising problems, fever and an increased risk of infection. Diagnosis is typically made by blood tests or bone marrow biopsy. Treatment may involve combination of chemotherapy, radiation, targeted therapy and bone marrow transplant in addition to supportive and palliative care [1]. The success of treatment depends on the type of leukemia and the age of the person since outcomes have improved in developed countries. In the United States, the average 5-year survival rate is 57% in adult and 60 to 85% in children below the age of 15. In children with acute leukemia who are symptom-free after 5 years, the cancer is unlikely to return [2].

Plant flavonoids have long been utilized for their anticancer, anti-oxidant, anti-apoptotic, anti-inflammatory, antimicrobial and immunodulatory activities [3]. The flavonoid-rich extracts of the following Philippine medicinal plants have been demonstrated to exhibit cytotoxic effects against leukemic cell lines: *Musa sapientum*, *Averrhoa*carambola, *Hylocereusundatus, Nepheliumlappaceum, Psidiumguajava*and *Punicagranatum.* The ethyl acetate leaf extract of *Pouteriacampechiana*, vernacularly known as "tiesa," contains 6 flavonoids that were found to arrest mitotic activities but were not further tested for any antitumor activities [4]. The fruit of "tiesa"was reported to have high flavonoid contents although these were not further screened for pharmacological activities. The present study seeks to compare the *in vitro* cytotoxic properties of the flavonoid-rich ethyl acetate of fruit extract of *P. campechiana* in both leukemic cancer cell lines and healthy human whole blood cells with the hope of exploiting anti-tumor potentials of these flavonoids.

II. Methods

2.1. Plant Extraction

Fresh ripe fruits of *P. campechiana*, harvested from Malolos (Bulacan), were washed with tap water to remove adhering dirt, peeled and the pulps mashed and homogenized to form a mass with soft palpable texture that was subsequently freeze-dried. Voucher specimen of the plant was submitted to the Philippine National Herbarium for authentication. The dried fruit pulp was macerated with 80% ethanol for 48 hours after which the solvent was evaporated under reduced pressure. The resulting crude extract was exhaustively defatted using

petroleum ether, acidified with 2M HCl and re-extracted with ethyl acetate and filtered. The filtrate was evaporated similarly to give an average yield of 1.84 w/v% of the ethyl acetate fraction of the fruit of *P. campechiana* which is designated in this study as EAFFPC which appears as a sweet, golden yellow semi-solid mass.

2.2. Screening for Flavonoids

EAFPC tested positive for flavonoids by responding to the WilstatterCyanidin test, which was manifested by a series of discoloration, and the Bate-Smith and Metcalfe's test, which was manifested by a strong red color. EAFPC was found to be freely soluble in water, acetone and 80% ethanol and sparingly soluble in ether and chloroform. FTIR analysis of EAFPC revealed the presence of benzopyrone nucleus and carbonyl carbons, alcoholic groups and unsaturation carbons with stretches at 1519 cm^{-1}, 1105 to 1025 cm^{-1} and 620 cm^{-1}, respectively, to reveal structures common to flavonoids. EAFPC was dissolved in water for injection to give a 1 mg/mL stock solution.

2.3. Cytotoxicity Assay

Human erythroid leukemic cell line (K562) and healthy human whole blood cells (HHWBC) were cultured in RPMI 1640 medium supplemented with 1 mM L-glutamine, 100 Units/ml penicillin and 0.1 mg/ml streptomycin, 10% inactivated FCS, and adjusted to pH 7.2 by the addition of 15 mM HEPES. The cells were maintained in a humidified incubator at 37^0C with 5% CO_2. Cells were cultured in 96-well plates (3×10^4 cells per well) containing 100 µL of the medium. Exactly 100 µL of water for injection (i.e., negative control), the aqueous EAFFPC (adjusted to deliver 10 to 60 μg of extract per well) or vincristine (10 µg per well) were added to the wells and plates were further incubated for 48 hours with 5% CO_2. After removal of 100 µL of the medium, MTT dye solution (5 µl, 5 mg/mL in PBS) was added and the plates were further incubated at 37°C for 4 hours with 5% CO2. After that, 100 µL of DMSO were added to each well and mixed thoroughly to dissolve the formazan crystals. The absorbance was measured using an ELISA plate reader at 570 nm with a reference wavelength at 630 nm. High absorbance readings corresponded to a high intensity of dye color, that is, to a high number of viable cells able to metabolize MTT salts [6]. Cell viability, in 5 replicate samples, was calculated thus:

$$\textbf{\% Cell survival} = \frac{\text{mean absorbance in test wells}}{\text{mean absorbance in control wells}} \times 100$$

III. Results And Discussion

Table 1 compares the cell viability of EAFFPC and vincristine upon *in vitro* exposure in leukemic and normal human whole blood cell lines. Results showed that EAFFPC exhibited a concentration-dependent cytoprotective effects on HHWBC in contrast to its cytotoxic effects in K562. Although no concentration-dependent cytotoxic effects were obtained in K562 cell lines, a well concentration of 60 µg demonstrated the greatest cytotoxicity which is much higher than the cell viability obtained with 10 µg of vincristine.

Table 1: Comparative cell viability of various concentrations of EAFFPC

Concentration Per Well	**Mean % Cell Viability**	
	K562 Leukemic Cell Lines	**Healthy Human Whole Blood Cells**
10 µg EAFFPC	175.8**	75.1
20 µg EAFFPC	403.3**	45.7
40 µg EAFFPC	415.7**	48.6
60 µg EAFFPC	55.4*	66.7
Vincristine = 10 µg	160.2	102.7

N = 5; *p < 0.001 vs vincristine; **p < 0.001 vs 60 µg EAFPPC

The significance of these findings are founded on the specific cytotoxicity of EAFFPC against leukemic cell lines which is beneficial since viability of HHWBC are not affected to suggest that it is not too cytotoxic even when administered in healthy humans. Furthermore, the comparable cell viabilities obtained with vincristine for both types of cell lines ($p > 0.05$) confirms the cytoprotective effects of EAFFPC on normal human blood cells. However, cell viability alone cannot be used as a sole parameter in assessing cytotoxicity against cancer cell lines. Mechanistic studies must be undertaken such as enzyme inhibitory assays, anti-oxidative pathways, modification of DNA architectural assembly, assessment of anti-apoptotic or antimitotic properties as well as anti-inflammatory and immunomodulatory evaluations utilizing marker compounds such as hormones, cell-membrane-bound peptides, cytokines, interleukins and alpha-tissue necrosis factors.

IV. Conclusions

This study confirms that the flavonoid-rich ethyl acetate fraction of the fruit extract of *P. campechiana*(EAFFPC) exhibit a highly-specific cytotoxicity on K562 leukemic cancer cell lines and a dose-dependent cytoprotective effects on HHWBC.

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