The Preliminary Cytotoxicity Study: Anti-Proliferative Activity of Aqueous Extract of *Clinacanthus nutans*, *Strobilanther crispa* and *Pereskia bleo* on Oral Squamous Carcinoma Cells ORL-48

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Background: This in vitro study was carried out to screen the aqueous extracts of selected local plants (*clinacanthus nutans*, *strobilanther crispa* and *pereskia bleo)* known locally to possess anticancer properties for cytotoxic activity. **Method:** The antiproliferative activity on human oral squamous cell carcinoma, ORL-48 was assessed based on a colorimetric assay using neutral red dye. **Result:** Clinacanthus nutans exhibited an IC_{50} of 49.8 ± 0.02 µg/mL on ORL-48 cells, while *Strobilanther crispa* and *Pereskia bleo* showed no antiproliferative response. **Conclusion:** The aqueous extract of *clinacanthus nutans, strobilanther crispa and pereskia bleo* does not possess anti proliferative activity towards oral squamous carcinoma cells ORL-48.

1. Introduction

Cancer therapies that utilizing the natural products is a promising approach where it suppresses, delay, reverse, or retard the process of carcinogenesis.[1] However, shifting from conventional therapy towards herbal cure would require empirical evidence with regards to efficacy and safety.[2]

In Malaysia the practice of traditional medicine is still very significant as many still rely on traditional preparations to heal various ailments. Cancer is readily perceived by the lay man to be associated with tumour and to the understanding of many tumours needs to be removed through surgical procedures. In order to avoid going through such invasive procedures, many have turn to alternative medicines to control and heal the disease.

Many plants from the tropical rain forest Malaysia have been used as traditional medicines to prevent and treat various types of cancers. *Clinacanthus nutans (Belalai gajah), strobilanther crispa (Pecah beling)* and *pereskia bleo (Jarum tujuh bilah)* are three plants traditionally used as cancer treatment and consumed in the form of decoction. Despite the availability of numerous anecdotal reports, evaluation of active anticancer effects has remained elusive. Therefore, this preliminary study was conducted to determine the antiproliferative activity of this plants.

2. Materials and Method

Oral cancer cell lines

Human oral squamous cell carcinoma (OSCC) cell line was used in the study. ORL-48 obtained from the Cancer Research Institute and Foundation, Subang Jaya Medical Centre (CARIF, Malaysia) was developed from a female patient with gum tumour. The cell line was cultured in DMEM F-12 medium (GibcoUSA) supplemented with 10% fetal bovine serum, 2 mL of penicillin-streptomycin and 1 mL of amphotericin B. Cell lines were incubated at 37°C in a humidified atmosphere containing 5% CO_2 (Thermo Forma).

Extracts preparation

The leaves of the plants were collected from Taman Botani Rimba Ilmu, University Malaya. The leaves were cleaned and dried. The leaves were then weighed and cut into small pieces before they were boiled in distilled water for several hours until the final volume is one third of the initial volume. The decoction obtained were centrifuged at 10,000 rpm to eliminate sediments. The decoction was concentrated and freeze-dried (EYELA FDU-1200, Tokyo) for use in the study.

Antiproliferative Assay

The antiproliferative activity of the extracts on ORL-48 cell lines was assessed based on a colorimetric assay using neutral red dye described in Zabidi et al.[3] The ORL-48 cells were seeded at 3 x 10^4 cells/mL in 96-well plates (Nunc, Germany). A 20 mg/mL stock extract of each plants was prepared using sterile distilled water

were diluted to varying concentrations of 0.1, 1.0, 2.5, 5.0, 7.5 and 10.0 µg/mL in separate wells using DMEM F-12 containing 10% fetal bovine serum, 1% penicillin-streptomycin, and amphotericin B. The cultures were incubated in a humidified incubator over a period of 72 hrs at 37 °C and 5% CO_2 (Thermo Forma, USA). Wells containing cells in the absence of the agents represented the negative control for the test. Following incubation, the culture medium was discarded and replaced with 100 μL neutral red (1% v/v). The culture plates were further incubated for 2 hours after which the cells were washed with 1 mL of solution containing 1% sodium dodecyl sulfate. The culture plates were placed on a rocker (Nunc, Germany) for 30 min and the density of the detached viable cells that absorbed the red dye was assessed based on the optical absorbance read using an ELISA microplate reader (Bio Tek, USA) at a wavelength of 540 nm. The concentration of extract causing 50% of cell death known as the inhibition concentration (IC_{50}) was determined by a graph of percentage of cell death versus concentrations of the plant extracts. Percentage of inhibition at every treatment concentration was calculated using formula stated below.

$$\% \text{ of Inhibition} = \frac{(\text{OD control} - \text{OD sample})}{\text{OD control}} \times 100$$

3. Result

Clinacanthus nutans exhibited an IC_{50} of 49.8 ± 0.02 µg/mL on ORL-48 cells. The extracts of *Strobilanther crispa* and *Pereskia bleo* were found not cytotoxic with non-determinable (ND) IC_{50} values.

Table 1. Cytotoxic activity of extracts on oral mucosal cancer cell line ORL-48 measured by the concentration required to inhibit 50% of cell proliferation (IC50).

Treatment	IC_{50} (µg/mL)
Clinacanthus nutans	49.8 ± 0.02
Strobilanther crispa	ND
Pereskia bleo	ND

4. Discussion

Screening of plant extracts for anti-proliferative activity was based on their IC_{50} towards ORL-48 cells. The neutral red cytotoxicity assay described by Zabidi et al.[3] was used to determine the IC_{50} of the extracts in causing 50 % cell inhibition. According to the standard National Cancer Institute criteria [4,5] crude extracts possessing an IC_{50} less than 20 µg/ml are considered active against the tested cancer cells. Thus, the aqueous extract of *Clinacanthus nutans* was not antiproliferative towards Human OSCC cell line with an IC_{50} >20 µg/ml, in agreement with Yong et al.[6] that reported poor antiproliferative effect of the methanol and water extracts of *Clinacanthus nutans*. *Strobilanther crispa* and *Pereskia bleo* were non-determinable (ND) because it does not reach 50% cell inhibition. Therefore, no value can be recorded.

Point that needs to be considered in work involving plant is the technique employed in the preparation of the compounds. The type of solvent used in the preparation is important as that will determine whether the compounds extracted from the plants are polar or non-polar. In this study, the preparation technique used was by aqueous extraction. This was based on the understanding that medicinal plants are often boiled, and the concentrates are then directly consumed by the patients. In other words, by simple boiling, whatever active compounds that were extracted from the plants have to be polar components which when consumed would be easily dissolved and absorbed by the body system.

5. Conclusion

Based on our findings, the polar compound extracted from aqueous extracts of *clinacanthus nutans*, *strobilanther crispa* and *pereskia bleo* does not have antiproliferative activities towards human OSCC cell line.

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7. References

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