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THE ROLE OF POLYAMINES IN ANTI-PROLIFERATIVE EFFECT OF SELECTED MALAYSIAN HERBS IN HUMAN LUNG ADENOCARCINOMA CELL LINE

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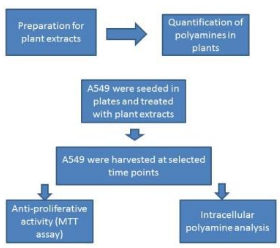
Graphical abstract



Abstract

Polyamine is a group of aliphatic amine that is necessary for cell growth and development. However, potential of developing cancer is enhanced with the increase of polyamine levels in the body. Since polyamines can be obtained through diet intake, it is essential for cancer patients to consume foods that have low level of polyamines. Therefore, this study aims to determine the polyamine level of selected plants; Sabah Snake Grass (*Clinacanthus nutans*), Daun Kadok (*Piper sarmentosum*) and Fig (*Ficus auriculata*). The antiproliferative effects of *C. nutans*, *P. sarmentosum and F. auriculata* were investigated using MTT assay and the polyamines content in these plants and intracellular content were quantified using High Performance Liquid Chromatography (HPLC). The results showed that polyamines content in *C. nutans* (41.49 nmol/g), *P. sarmentosum* (55.01 nmol/g) anf F. auriculata (39.28 nmol/g) were classified under low level. The IC_{50} values for these plants were in the range of 20-30 mg/ml. This study demonstrated that these plants inhibit the A549 cell's growth after 24 hours to 96 hours of exposure. Depletion of intracellular polyamine after 48 hours to 96 hours of exposure was also identified. In conclusion, the study showed that *C. nutans, P. sarmentosum* and *F.auriculata* contain low level of polyamine and have anti-proliferative effect against A549 cells. Therefore these plants are recommended to reduce or delayed the occurrence of malignancy and prevention of cancer recurrence after successful treatment.

Keywords: Cancer, A549, polyamines, natural product, growth inhibition

1.0 INTRODUCTION

Cancer, also known as malignancy is characterized by uncontrollable cell growth. Cancer cells progressed when normal cells do not undergo programmed cell death (apoptosis) and continue to grow out of control. Genetic factor, lifestyle factors including diet, physical activity, obesity, smoking, environmental exposures to different types of radiation and chemicals (carcinogen) and certain types of bacterial and viruses' infection are several causes of cancer. The most common type of cancer is lung cancer [1]. In addition, 26% of all female cancer deaths and 28% of all male cancer deaths in 2013 were caused by lung cancer in the United States [1]. The occurrence of lung cancer has increased rapidly since the beginning of 20th century.

Interest among scientist regarding the link between polyamines and cancer has increased recently. Polyamines are vital in maintaining human health because they performed functions that are necessary for cell development [2]. However, increasing intracellular polyamines content could lead to potential cellular poisons to the body such as carcinogenesis and tumor growth since it promotes cell proliferation and growth [3]. Moreover, higher levels of polyamines have been found in various types of cancer cells [2]. Since polyamines are increased in cancers cells, they have become the target for potential chemotherapeutic agents [4, 5].

Cancer chemoprevention is one of the approaches to decrease the rate of cancer cases. Occurrence of malignancy can be reduced or delayed using cancer chemoprevention that may involve administration of a natural, synthetic or biological agent. It may also interrupt several steps in tumor initiation, promotion and progression [6]. Current cancer therapies such as chemotherapy, radiotherapy and radical surgery can result to undesired physical and psychological distress to the patients. Therefore, cancer chemoprevention which provide high therapeutic efficacy with fewer side-effects as compared to the current anti-cancer drugs in the market are required. During the last four decades, the use of natural product has greatly increased because of the belief that they are non-toxic and provide superior therapeutic effect as compared to established drugs [7, 8, 9]. Therefore, this study was focused on three medicinal plants in Malaysia that are well known with anticancer property namely Sabah Snake Grass (*Clinacanthus nutans),* Daun Kaduk *(Piper sarmentosum)* and Fig *(Ficus auriculata)*.

The general aim of this recent study is to investigate the role of polyamines in cancer preventive effect induced by *C. nutans*, *P. sarmentosum* and *F.auriculata*. To achieve this, there are two parts of this study which include the process of polyamine extraction and determination in chosen plants, Sabah Snake grass or *C. nutans* and Daun Kadok or *P. sarmentosum.* It is believed that in order to prevent the risk of getting cancer, ones should consume foods that have low polyamines content. The second part is *in vitro* assessment which aims to investigate the anti-proliferative effect of these selected plants against lung cancer cell (A549). To confirm the polyamines play role in this chemo preventive effect, the intracellular changes induced by *C. nutans* and *P. sarmentosum* in polyamine content in cancer cells were quantified.

2.0 EXPERIMENTAL

2.1 Sample Preparation

The leaves of these plants were collected and rinsed with tap water followed with distilled water. Next, the leaves were blended using electrical blender and packed in bags and stored in -80°C freezer. Then, samples were freeze dried. After that, dried leaves were powdered using electrical blender, packed in bags and stored in room temperature up to a maximum of four weeks.

2.2 Quantification and Classification of Polyamines in *C. nutans, P. sarmentosum and F. aucilurata*

The determination for polyamine concentration from *C. nutans, P. sarmentosum* and *F.auriculata* was done by dissolving the dried organic residues resulted from evaporation in 200 µl acetonitrile and vortexed rapidly. The solution were centrifuged at 13 000 rpm for 3 minutes and transferred to HPLC vials (70 µl). Samples were analysed by reverse-phase HPLC on a Hichrom RPB 5 µm column using a gradient of 100% acetonitrile to 40:60 (v/v) acetonitrile/water. The classification is based on the method by [10].

2.3 In Vitro Analysis of Anti-Proliferative Effect

The A549 cells, a human lung adenocarcinoma cell line, were grown in Dulbecco's Modified Eagle Medium (DMEM) with a high glucose and L-glutamine, 10% (v/v) Fetal Bovine Serum, and 1% (v/v) Penincilin-Streptomycin. The cells were grown at 37°C in a humidified atmosphere flushed with 5% CO_2. Cells were routinely subcultured every 2-3 days, and were seeded at 3.2 x10^3 and grown for 48 h prior treatment.

The MTT assay was carried out to determine IC_{50}. Cells were grown on 96-well microtitre plates for 48 h and exposed to the appropriate concentration of plant extract .After 48 h exposure, 10 µL of a 5 mg/ml sterile solution of MTT in complete PBS was added to the cells. Plates were then left for 4 h in a humidified atmosphere of 5 % CO2 at 37◦C. Actively respiring cells metabolise MTT to an insoluble formazan salt which was then dissolved using 100 % DMSO. After 20 min, the plate was read at a reference wavelength of 540 nm and a test wavelength of 690 nm and the results were expressed as a percentage of control values.

2.4 Measurement of Intracellular Polyamines

After appropriate time of plant extract exposure, A549 were harvested by the removal of cells and medium into 15 ml centrifuge tubes. Next, the cells were centrifuged at 2800 $_{gav}$ for 5 minutes. The supernatant was discarded and the pellet was washed with 1 ml of PBS. The cell suspension was centrifuged again at 7500 ***g***avfor 5 min and the supernatant discarded before resuspending the pellet in 300 µl of 0.2 M PCA. This was stored at −20◦C until analysis by HPLC. The method of HPLC used was that of pre-column dansylation. Samples were dansylated at 25◦Covernight, extracted in toluene, blown to dryness in a nitrogenstream, and then reconstituted in 200 µl of acetonitrile. Samples were analysed by reverse-phase HPLC on a Hichrom RPB 5 µm column using a gradient of 100% acetonitrile to 40:60 (v/v) acetonitrile/water.

3.0 RESULTS AND DISCUSSION

Recent investigations done by [11], revealed that increased polyamine availability enhances the capability of tumor cells to invade and metastasizes to a new tissue while diminishing immune cells' anti-tumor immune function. Human need enough polyamines to help growth and healing, but not in excessive amount as it can slow down the immune systems and change the metabolism of the tissues [12]. It has been stated that external dietary source provides a larger quantity of polyamines than the endogenous biosynthesis. The latter produces about only 1–2 nmol of putrescine per hour per gram of tissue in the most active organs [13].

Table 1 Classification and total polyamine content in *C.nutants*, *P. sarmentosum* and *F. aucilurata*

Samples	Total Polyamine (nmol/mg protein)	Classification
C. nutans	41.49 ± 7.71	Low
P. sarmentosum	55.01 ± 0.06	Low
F.aucilurata	39.28 ± 3.01	Low

In contrast, dietary source can to a certain extent regulate biosynthesis of polyamines. Since one of the main sources of polyamines exist and maintain in organisms is through food intake (diet), it is crucial to tightly control the polyamine content inside the body at a normal range of concentration by consumed food with low level of polyamines. Table 1 showed the total polyamine concentration (nmol/mg protein) measured in the three medicinal plants. Based on [7], the total polyamines detected were classified into one of these three classes either low (less than 100 nmol/g/ml), intermediate (101-200 nmol/g/ml) and high (more than 200 nmol/g/ml). Therefore the polyamine content in the plants in this study was classified under low level.

This finding indicates that these plants are suitable to be proposed for nutritional cancer therapy. Eating the right kinds of foods before, during and after treatment can help the patients feel better and stay stronger. Nutritional cancer therapy is not only important for the primary prevention of cancer but also for the prevention of cancer recurrence, which is of utmost importance in determining survival [13]. Moreover, it is very essential to take low polyamine diet among cancer survivor and for preventive action because low polyamine diet can maintain the level of polyamine inside the body at low or normal concentration. For example, mice fed experimental chow containing high concentrations of polyamines for 26 weeks had significantly higher concentrations of blood spermine and spermidine than mice fed chow containing low or normal concentrations of polyamines [14]. These current findings are also supported by another study which revealed that blood spermine concentrations of healthy male was significantly increased due to the long term consumption of natto, but the concentrations remained unchanged in control group. Therefore, reduction of high polyamine intake seems to be an important target for polyamine-based cancer therapy particularly because inhibition of polyamine synthesis alone failed to produce a favorable effect on cancer treatments in several clinical trials [15].

In order to further validate their effect as anti-proliferative agents, it is crucial to investigate the growth inhibition effect induced by these plants in A549 cells using MTT assay. Based on Table 2, IC_{50} values obtained after 48 hours exposure for these three plants was in the range of 20-30 mg/ml.

Table 2 Summary of IC_{50} values after 48 hours exposure for *C. nutans* and *P. sarmentosum* in A549 cells

Samples	IC_{50} value
C. nutans	20 mg/ml
P. sarmentosum	30 mg/ml
F. auriculata	25 mg/ml

The intracellular polyamines of untreated and treated cells were determined and compared at the end of experiment to see the effectiveness of *C. nutans, P. sarmentosum* and *F. auriculata* in reducing the polyamine content of A549 carcinoma cells. The control samples showed successive increased in amounts of putrescine, spermidine and spermine after 72 hours and 96 hours. However, amounts of individual polyamines were slightly decreased after 72 hours and 96 hours in treated cells (Figure 1). This study proved that *C. nutans, F. auriculata* and *P. sarmentosum* were effective in reducing the amount of polyamines in A549 carcinoma cells.

Figure 1 showed treatment of A549 carcinoma cells with 20 mg/ml *C. nutans* and 30 mg/ml *P. sarmentosum* results in depletion of intracellular polyamine after 72 hours until 144 hours of exposure. This finding showed that the plants also have an additional effect on the polyamine metabolism itself. These plants seemed to have an additional mode of toxicity by depleting intracellular polyamines. It is believed that antiproliferative action of a drug is mediated by depletion of polyamine level. Because polyamines are the requirements for cell growth, interference of polyamine biosynthesis has been a promising therapeutic approach to the identification of antiproliferative drugs with chemotherapeutic potential.

It is also noted that intake of food that contained low level of polyamine may induce inhibition of polyamine synthesis and may have favorable effects on cancer therapy [15]. Findings from this study proved that *C. nutans, F. auriculata* and *P. sarmentosum* are effective in reducing the amount of polyamines in A549 cancinoma cells.

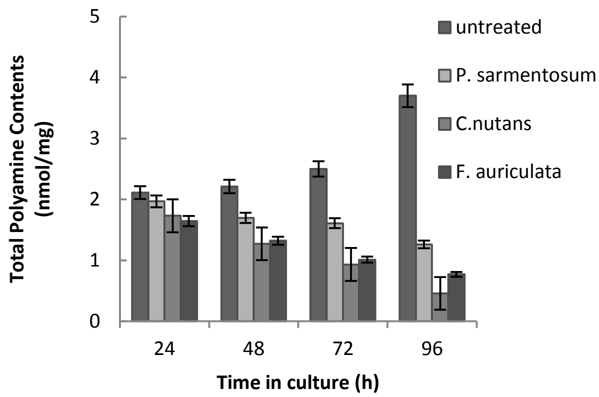


Figure 1 Total intracellular polyamine content of untreated and treated A549 cell with polymanines of *C. nutans, F. auriculata* and *P. sarmentosum*

4.0 CONCLUSION

Clinacanthus nutans, Ficus auriculata and *Piper sarmentosum* contain low level of polyamine and have anti-proliferative effect against lung cancer cell (A549). Low levels of polyamines in these plants are essential to maintain the level of polyamine inside our body at low or normal concentration especially for cancer survivors. Moreover, IC_{50} value were also potent enough to cause the inhibition of cell growth. These findings suggested that *C. nutans, F. auriculata* and *P. sarmentosum* could be consumed to reduce or delay the occurrence of malignancy and may help in preventing cancer recurrence after successful treatment.

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