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Anti-Proliferative Activity of Swertia chirayita (Roxb. Ex Fleming) Karst Crude Extracts on Different Cancer Cell Lines

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Abstract

A number of phytogenic compounds derived from medicinal plants have been studied for medical development. Among them, *Swertia chirayita* (Roxb. ex Fleming) karst, also known as *Chirayito*, an herb cultivated in high hills of Nepal, has been selected to investigate the anticancer property. The aim of the present study is to screen and evaluate the anticancer potential of *Swertia chirayita* (Roxb. ex Fleming) Karst extract against human biological cancer cell line; colon CACO-2, brain KELLY and breast MCF-7 respectively. The methanolic extracts of *Swertia chirayita* demonstrated anti-cancer activities in compare to positive control drug 5' fluro uracil which were evaluated by assessing the effect on cell viability by MTT assay and fluorescence microscopy. The crude extracts exhibited selectively cytotoxic *in vitro* to KELLY, MCF-7 and CACO-2 cells. The effect of 1000 µg/mL crude extract was equivalent to 1.32 µg/mL of 5-fluorouracil in MCF-7 cell to inhibit 66.21% cell whereas effect of 1000 µg/mL extract was equivalent to 2.43 µg/mL of same drugs in KELLY cell to inhibit by 38.83% and finally 1000 µg/mL of the same extract was equivalent to 2.94 µg/mL of drug to inhibit 38% CACO-2 cells. The IC50 of 5 Fluro uracil (5-FU) in MCF-7, CACO-2 and KELLY was measured which was 2.24 µg/mL, 2.23 µg/mL and 1.58 µg/mL respectively obtained by plotting a measure of cell death rate vs the concentration of the drug. So, its extract is recommended in therapeutic form accordingly. The study concludes that the plant extract may be sensitive, specific and cost effective biomarkers in early prevention of breast, colon and brain cancer assessing response to treatment.

Keywords: *Amarogentin; Cancer Cell Lines; Extract; MTT Assay; Swertia chirayita*

Introduction

Traditional medicine and its relevance to public health have received a great deal of attention in developing countries [1,2]. According to World Health Organization (WHO), more than 80% of the people around the world use plant extracts or their active constituents as folk medicine in traditional therapies [3]. Plants exhibit versatility in the synthesis of metabolites that could be therapeutic significance in the research field of medicines [4]. Dietary supplements along with plant extracts like flavonoids, phenolic and glucosinolates compound have been found as major antioxidant and anti-carcinogenic potential [3,5]. Many experiments are being done to isolate active components from medicinal plants for their possible utilization in non-communicable disease treatment like cancer. Natural plant products have been contemplated of exceptional value in the formulation of effective anti-cancer drugs with less possible host cell toxicity [6]. The investigation of anti-cancer drugs from natural plant resources has been emphasized in order to find useful drugs for malignancies. It also has been proposed sequential use of cytotoxic agents could improve anticancer therapy by inhibiting tumor cell proliferation and phenotypic diversification [7]. Over 50% of the drugs in clinical experiments for anti-tumor activity were already isolated from natural sources [8]. The advantages of using traditional medicine include dissimilarity, flexibility, easy to approach, relatively low cost, low levels

of technological input and increasing economic importance [5]. There are reports on more than 150 plant herbs still being used all over the world in daily life to treat different diseases [9]. Traditional medicine and its relevance to public health have received a great deal of attention in developing countries [10,11]. Out of an estimated 9,000 species of plants found in the eastern Himalaya as a whole, 39% are endemic to this mountain range [12-15]. Nearly 7,000 species of higher plants are found in Nepal, of which 5% are endemic and 10% are medicinal and aromatic. With 75 vegetation types ranging from dense tropical forests to alpine vegetation that covers over 50% of the total geographical area of the country forms the land resource base for the provision of medicinal and aromatic plants. In view of this nature, it was planned to examine the *in-vitro* anticarcinogenic effect of methanolic crude preparation of *Swertia chirayita* with an objective of substantiating the validity of its work.

Plant profile

a. Kingdom: *Plantae*
b. Phylum: *Angiosperms*
c. Class: *Asterids*
d. Order: *Gentianales*
e. Family: *Gentianaceae*
f. Genus: *Swertia*
g. Species: *chirayita*

Among diverse aromatic medicinal plants, *Swertia chirayita* (Roxb. ex Fleming) karst is a natural herb belonging to family *Gentianaceae*. The herb share similarity with hops in two aspects. a) Its bitterness potential. The bitterness of this plant is attributed to chiratin, which on hydrolysis yields two bitter principles, ophelic acid and chiratin [16] b) The herb is reported to show antimicrobial action against some gram positive and gram negative micro-organisms [17,18]. Besides, the plant extract has been reported to possess antipyretic, anti-viral [19], anti-helminthic, anticarcinogenic [20], hepatoprotective [21], hypoglycemic activities. Earlier studies documented the presence of flavonoids, xanthones, terpenoids, iridoid and secoiridoid glycosides in the *S. chirayita* plant [22].

Materials and Methods

Materials

The dry stem, root and leaves of *Swertia chirayita* were made powdered mixing 1:1:1 and then extracted using solvents Methanol by Cold method. The other material are Filter stritops (0.22 µm), Falcon tubes (15 mL), Culture flasks (25 cm^2), Multichannel Serological pipettes (5 mL), sterile pipette tips, 5-fluorouracil (5-FU, Anticancer drugs), fetal bovine serum albumin, Triton-X 100, and Hank's Balanced Salt Solution (HBSS), Complete Eagle's Minimum Essential Medium (EMEM), Complete RPMI 1640 media, Cancer Cell lines CACO-2, MCF-7 and KELLY, Tetrazolium dye and 7, 12-dimethylbenz (a) anthracene (7,12-DMBA), DMSO, Distilled water Incubator (37^0C), Laminar flow hood, Refrigerator, Florescent microscope, Driers, Beakers, Grinders, Eliza Reader, 96- Well plate, Assay Buffer, MTT Reagent, Vortex, Centrifuge, Filter papers, Funnel, Measuring cylinder, Water bath, Magnetic stirrer and Separating funnel.

Methods

Sample Collection

The plant *Swertia chirayita* was collected from Rasuwa districts of Nepal. All plants were collected at the end of the flowering season in late August to October 2017 when the plants were in the seed dispersal phase. The specimens were checked and identified as *Swertia chirayita* (Roxb. ex Fleming) karst by Senior Botanist Mr. Binod Basnet at National Herbarium and Plant Laboratories, Godavari (Lalitpur) under the Department of Plant Resources (DOPR) Kathmandu.

Sample preparation

The root, shoot and leaves were separated, washed thoroughly with tap water and shade dried at room temperature of 24 - 30oC. These different parts of the plants were grounded in the spice grinder (Spark machines, manufactured under quality system, ISO -9001:2000, India). The root, leaf and stem powdered were mixed at 1:1:1 for solvent extraction.

Extraction

The cold extraction was done using 40g of the dried powdered plant sample in a beaker mixed with 250 mL HPLC grade Methanol CH_3OH = 32.04, Qualigens, Fisher Scientific, CAS No 67, 56-1 and stirred for 30 minutes. The mixture was kept in room temperature for 48 hours. After 48 hours, the solution was filtered using filter paper. The extract was then dried using rotary vapour at Nepal Agricultural Research Council (NARC) Khumaltar, Lalitpur.

Cell line and culture

MCF-7, CACO-2 and KELLY cell lines from human carcinoma of the breast, colon and brain respectively (provided by Shikhar Biotech Nepal Private Limited) were used to study anti-proliferative effect (Oyama and Eagle, 1956). The cell line was maintained in a 75-ml plastic tissue culture flask (Nunc, Intermed Co. Ltd., Copenhagen, Denmark) in EMEM medium supplemented with 10% heated fetal bovine serum and maintained at 37°C with 5% CO_2 atmosphere and 95% humidity.

Cytotoxic assay

The cells were seeded in a 96-well plate at a density of 1000 cells/well. Following a 24 hrs incubation, cells were treated with varying concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.625 and 7.8 µg/ml) of crude extract respectively. Anticancer drug named 5-FU was taken as positive control and negative control (without drugs and extracts) to measure the effect of relative ratio (%).

MTT Assay

The MTT assay is a test of metabolic competence based upon assessment of mitochondrial performance, relying on the conversion of a yellow dye 3-(4,5-dimethyl-2- thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to the purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells [23]. The cell lines of human breast cancer (MCF-7) and brain tumor (KELLY) with RPMI-1640 medium; and Colon cancer (CACO-2) were incubated with EMEM medium, supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U/mL streptomycin and 100 U/mL penicillin at 37°C with 5% CO2. For experiments, cells were plated in 12-well plates. After 24 hr, the extracts dissolved in DMSO were added. Cells were also treated with DMSO only in amount equivalent to that of extracts and control consisted of only media. At the end of 72 hr incubation, the medium in each well was replaced by fresh medium containing 0.5 mg/mL of MTT. Three hours later, the formazan product of MTT reduction was dissolved in Isopropanol, and absorbance was measured using a ELISA Reader at 492 nm. The effect of extracts was determined as the percentage of reduced cell in the control samples also at the same wave length 492 nm [23,24]. Results were expressed as average of triplicates.

Results and Discussion

Swertia chirayita is one of the highly traded medicinal plants of Nepal. A number of phytogenic compounds derived from medicinal plants have been studied for medical development. Among them, *Swertia chirayita* (Roxb. ex Fleming) karst, also known as *Chirayito*, an herb cultivated in high hills of Nepal, has been selected to investigate the anticancer property. The present study showed that this plant has anticancer potential against human cancer cell line; colon CACO-2, brain KELLY and breast MCF-7 respectively by assessing its effect on cell viability by the MTT assay and fluorescence microscopy. The results demonstrated that potent anti-carcinogenic compounds are present in *Swertia chirayita* and its extract may be used in early prevention of breast, colon and brain cancer. In Ayurvedic medical practices *Swertia chirayita* is one of the oldest medicinal herbs that were used for the treatment of liver disorders [25] but the anti-carcinogenic potential has still remained unexplained. The aqueous and amarogentin rich crude extract of the plant was first studied and reported to have anti-carcinogenic activity on mouse skin which was induced DMBA [26].

The amarogentin rich fraction of *Swertia chirayita* showed to inhibit cell proliferation and enhance apoptosis, which are major physiological process regulating tumour formation, growth and spread [20,27]. In correlation to our previous research done in *Swertia chirayita* of Rasuwa District in Nepal [28], Quantities of amarogentin, a secoiridoid glycoside ([(2S, 3R, 4S, 5S, 6R)-4, 5-dihydroxy-6-(hydroxymethyl)-2 [[(5S)-5-hydroxy-1oxo-4, 4a, 5, 6-tetrahydro-3H-pyrano [3, 4-c] pyran-6-yl]oxy] oxan-3-yl] 2, 4-dihydroxy-6-(3-hydroxyphenyl) benzoate) were found to be highest in aqueous and ethanol leaves extracts (0.28 and 0.23 mg/g DW) from Goljung (Village Development Committee of Rasuwa) and lowest in aqueous and ethanol root extracts (0.007 and 0.002 mg/g DW) from Gallang (Village Development Committee of Rasuwa). So the presence of amarogentin in crude extract could be found to have cytotoxic action on MCF-7, KELLY and CACO-2 cells, indicating it as anti-carcinogenic agent. The figures 1, 2 and 3 show the IC_{50} of 5 Fluro Uraccil (5 FU) to inhibit growth of selective cells. The IC_{50} of 5-FU in MCF-7, CACO-2 and KELLY was measured which was 2.24 µg/mL, 2.23 µg/mL and 1.58 µg/mL respectively obtained by plotting a measure of the cell death rate vs the concentration of the drug. So, its extract is recommended in therapeutic form accordingly. The effect of crude extract has been shown in figure 4. The effect of 1000 µg/mL crude extract was equivalent to 1.32 µg/mL of 5' fluro uracil in MCF-7 cell to inhibit 66.21% cell whereas effect of 1000 µg/mL extract was equivalent to 2.43 µg/mL of same drugs in KELLY Cells to inhibit by 38.83% and finally 1000 µg/mL of the crude extract was equivalent to 2.94 µgm/mL of drugs to inhibit 38% CACO-2 cells. So the results demand special attention for its varied role in human health. It reveals newer medicinal properties to focus on their potential pharmaceutical application especially for cancer prevention as it holds both in therapeutic and preventive medicines.

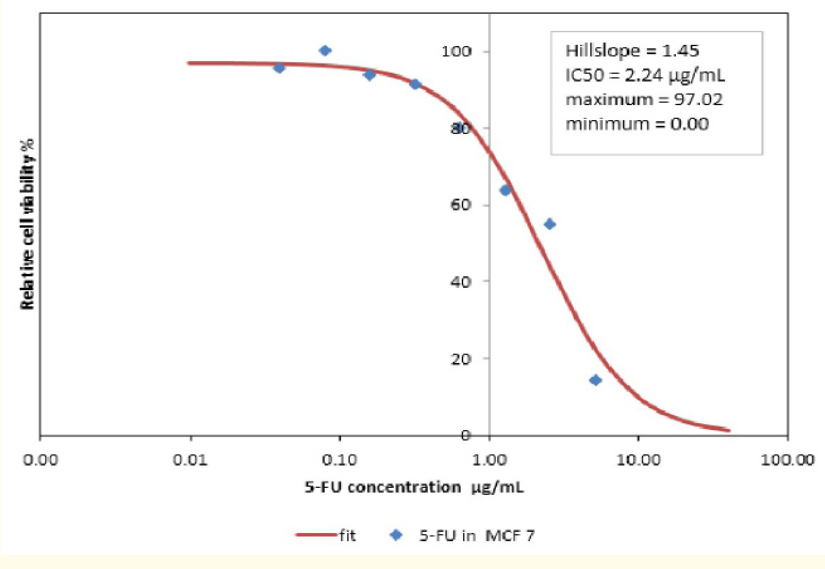

Figure 1: *Effect f Crude extract equivalent to drug in MCF-7.*

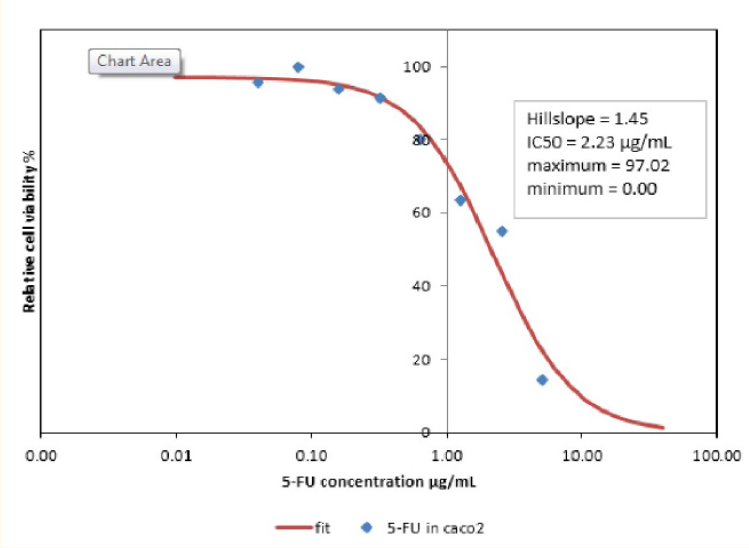

Figure 2: *Effect f Crude extract equivalent to drug in CACO-2.*

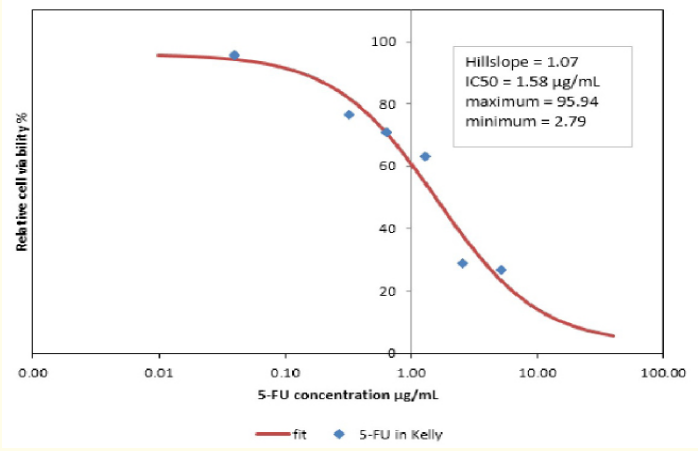

Figure 3: *Effect f Crude extract equivalent to drug in KELLY.*

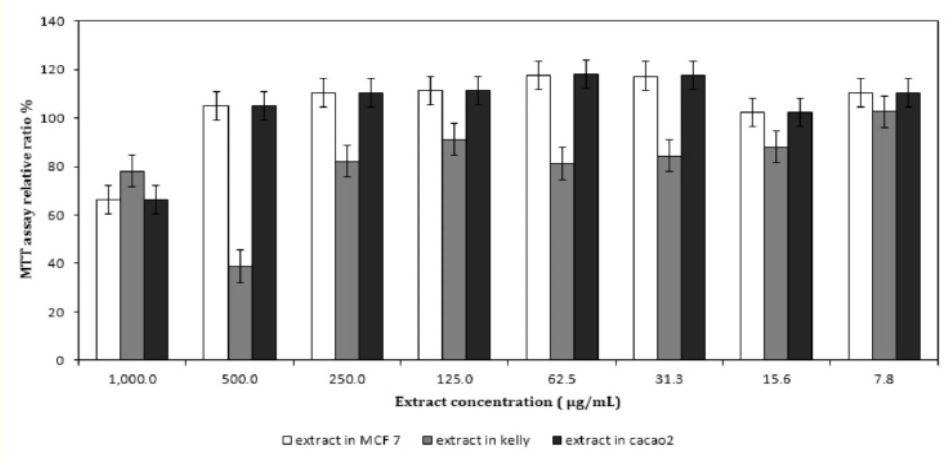

Figure 4: *Effect of Swertia Chirayita crude extract and drug on cancer cell lines.*

Conclusion

We have been able to show that crude extract of root, steam and leaf of *Swertia chirayita* contains different compounds that have health benefits. The amount of Alkaloid is high as expected because the plant contains Swertiamarin, Mangiferin and Amarogentin along with other phytochemicals. Also the plant is reported to have antioxidant properties, antibacterial against major pathogens. In correlation to our previous research conducted [28,29], the results elucidate *Swertia chirayita* has potential anticancer properties in MCF-7, KELLY and CACO-2 cells and a detailed further investigation is suggested.

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