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# In Vitro Cytotoxic Effect of Rodgersia Sambucifolia (Hemsl) in the Treatment of Leukemia

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***Abstract*— Rodgersia sambucifolia (Hemsl) commonly known as rhizome of Featherleaf, Rodgers flower is one of the Chinese traditional medicinal plants in cancer therapy. The powder of R. sambucifolia rhizome was traditionally used by Chinese traditional healer for the treatment of leukemia and throat cancer. However, no scientific research has been carried out about this traditional claim. The aim of the present study was to investigate the potential cytotoxic effect of this plant rhizome extract against human T4 lymphoblastoid cell lines (CEMss). Among the ethanol and water extracts of R. sambucifolia, the ethanolic extract showed a significant (P<0.01) anti-proliferative effect which was followed by water extract at a concentration of 3.0 and 10.8 µg/ml subsequently. The ethanol and water extract of the R. sambucifolia reveals the presence of alkaloids, carbohydrates and glycosides, tannins and phenolic compounds, flavones and flavonoids and the absence of proteins and amino acids, sterols, fixed oils and fats. The present study opens and new stand point to validate R. sambucifolia extracts as one of the cytotoxic drugs for cancer therapy based on the traditional claim.**

***Index Terms*— Rodgersia Sambucifolia, Leukemia, In-Vitro, Ethanolic Extract, Water Extract, CEMss**

## I. INTRODUCTION

Cancer is an abnormal growth of cells spreading unusually throughout the body in different conditions and environments. Cancer cells have the ability to multiply constantly, contiguous infiltration of tissues, and invade to other organs through the blood and lymph [1]. Cancer is a leading cause disease worldwide. Population of cancer in Malaysia is expanding every year, there are around 90-100,000 individuals are living with cancer [2]. Leukemia is a condition in which there is an abrupt increase in the production of white blood cells. These excessive white blood cells are mostly immature. Leukemia also causes anemia due to reduced production of other blood cells. Leukemia is classified into major classes and sub classes depending upon the production of blood components such as red blood cells and platelets. Leukemias are classified into various types and are abbreviated for ease of referencing. The classification is based upon the blood cells affected [1]. Ionizing radiations, inhalation of carcinogens such as benzene and viral infections consider being the major hazard to cause leukemia [3].

Herbal medicine was used for decades by ancestors to treat various health conditions. Herbal medicine is one of the most generally utilized complementary and alternative therapies (CAM) by individuals with cancer. It was found that people with cancer particularly takes herbal medicines to avoid adverse effects caused by modern medicines.

Rodgersia sambucifolia Hemsl (Family: Saxifragaceae) is commonly known as Bloody Mary in English, Memali in Malay and Hong Jiang or Jiu Yue Yan Tuo in Chinese. R. sambucifolia is normally cultivated on mountains. It can grow till 60 cm (2 feet) tall, with small white flowers rapidly aging to brown-green. The rhizomes are thick which have fibrous roots and the rhizomes spread just under the surface of the soil. It prefers constantly wet soil and cool temperatures. It was claimed by traditional healers as an astringent and for the treatment of cough, inflammation, pain, rheumatism, bruises, fractures, rheumatoid arthritis, bleeding wounds, dysentery, diarrhea as well as to regulate menstruation [4].

There was no scientific evidence for the cytotoxic activity of R.sambucifolia in the treatment of leukemia. These observations directed the present scientific investigation to evaluate the traditional claim of R.sam*bucifolia* in leukemia.

## II. MATERIALS AND METHODS

### A. Plant materials

The rhizomes of Rodgersia sambucifolia (2.5 kg) were collected from Labu Batu 8, Negeri Sembilan, Malaysia in the month of May 2016. The collected plant was carefully examined and authenticated by a pharmacognosist, KPJ Healthcare University College, Nilai, Negeri Sembilan, Malaysia (Ref. NO: KPJUC/CRI/PA/2016 (02)).

### B. Extraction

The collected rhizomes were washed thoroughly with running tap water to remove the adherent materials and dried in hot air oven at 50$^{o}$c. Then it was pulverized mechanically into coarse powder. The coarsely powdered rhizome of *Rodgersia sambucifolia* (1 kg) was taken in an aspirator bottle and extracted successively by cold maceration technique with solvents such as ethanol and water respectively for 6 days. At the end of each extraction, there were filtered through Whatmann filter paper. After filtration, solvents were evaporated from the extracts using rotatory

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vacuum evaporator [5]. The color, consistency, and percentage yield was recorded in Table 1.

Table 1 The colour, consistency and percentage yield of different extracts of *Rodgersia sambucifolia*

| Extracts | Colour | Consistency | % yield |
|---|---|---|---|
| Ethanolic | Yellowish brown | Powder | 4.14% |
| Water | Brownish | Mucilaginous | 43.12% |

### C. Preliminary Phytochemical Screening

All the extracts of *R. sambucifolia* were subjected to preliminary phytochemical analysis to determine the phytoconstituents which may be responsible for cytotoxic activity. The phytochemical test was carried out for all the extracts to determine the phytoconstituents such as alkaloids, carbohydrates and glycosides, proteins and amino acids, sterols, fixed oils and fats, phenolic compound and tannins, flavonoids, saponins, gums and mucilage by standard procedure [6].

### D. Thin Layer Chromatography (TLC) profile

Qualitative TLC parameters of ethanol and water extracts of rhizome of *R. sambucifolia* were investigated by using silica gel G plate as stationary phase, butanol:acetic acid:water (4:1:5), HCl:acetic acid:water (3:30:10) and phenol:water (3:1) as mobile phase. The spots were visually detected in an iodine chamber [7]. Results were recorded in Table 2.

Table 2 TLC profile for ethanolic and water extract of *Rodgersia sambucifolia*

| Test samples | Solvent system | $R_f$ value |
|---|---|---|
| Ethanolic extract | Butanol:acetic acid: water (4:1:5) | 0.22<br>0.48 |
| Water extract | Butanol:acetic acid: water (4:1:5) | 0.33<br>0.52<br>0.64 |
| Ethanolic extract | HCl:acetic acid:water (3:30:10) | 0.52 |
| Water extract | HCl:acetic acid:water (3:30:10) | 0.19<br>0.60 |
| Ethanolic extract | Phenol:water (3:1) | 0.23<br>0.52 |
| Water extract | Phenol:water (3:1) | 0.15<br>0.39<br>0.68 |

### E. Cytotoxic activity of R. sambucifolia

The study was carried out by UPM – MAKNA Cancer Research Laboratory, Institute of Bioscience, University Putra Malaysia Serdang. The Human T4-lymphoblastoid cell line (CEMss) was grown in Dulbeccos Modified Eagles Medium containing 10% fetal bovine serum (FBS) and was maintained at 37oc, 5% CO2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the extracts. They were initially dissolved in dimethylsulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. One hundred microliters per well of each concentration were added to plates to obtain final concentrations of 500, 250, 125, 62.5 and 31.25 µg/ml. The medium containing without samples were served as control. Triplicates were done for all concentrations. After 48 h of incubation, 15 µl of MTT (5 mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37 0C for 4 h. The medium with MTT was then be flicked and the formed formazan crystals were solubilized in 100 µl of DMSO and then measure the absorbance at 570 nm using micro plate reader. The concentration which inhibited 50% of cellular growth ($IC_{50}$ value) was also determined [8].

### F. Morphological Studies

Morphological studies by using the normal inverted microscope was carried out for the extracts of rhizome of R. sambucifolia. The Human T4-lymphoblastoid cell line CEMss were treated with both extracts for 48 h and the morphological changes in the cell lines were observed, where untreated cells served as negative control.

Morphological characterization was also examined by using fluorescence microscope. After treatment, the cells were stained with a 1:1 mixture of Acridine orange (AO) 100 µg mL-1 and Propydium iodide (PI) 100 µg mL-1 for 2 min. 10ml of the suspension was placed onto a glass slide and examined with a fluorescence microscope. In addition, morphological changes were also identified by Scanning electron microscope [9].

## III. RESULTS

The colour, consistency and percentage yield of R. sambucifolia are tabulated in Table 1. Among the two extracts, water extract had the highest percentage yield which was 43.12% whereas ethanol extract had a percentage yield of 4.14%. The phytochemical analysis done for both extracts revealed the presence of alkaloids, carbohydrates and glycosides, tannins and phenolic compounds and flavonoids. The TLC profile for both extracts is tabulated in Table 2. Ethanolic extract of R. sambucifolia showed the presence of 2 spots with Rf values of 0.22 and 0.48 at the mobile phase of butanol:acetic acid:water (4:1:5) out of 2 spots with Rf value 0.22 is major. Water extract showed the presence of 3 spots at the same mobile phase with the Rf values of 0.33, 0.52 and 0.64. Ethanolic extract of R. sambucifolia showed the presence of 1 spot with Rf value of 0.52 at the mobile phase of HCl:acetic acid:water (3:30:10). Water extract showed the presence of 2 spots at the same mobile phase with the Rf values of 0.19 and 0.60. Ethanolic extract of R. sambucifolia showed the presence of 2 spots with Rf values of 0.23 and 0.52 at the mobile phase of Phenol: water (3:1) out of 2 spots with Rf value 0.52 is major. Water extract showed the presence of 3 spots at the same mobile phase with the Rf values of 0.15, 0.39 and 0.68.

In vitro cytotoxicity for both extracts against Human T4-lymphoblastoid cell line (CEMss), with the comparison of non-cancerous human peripheral blood lymphocytes (PBL) was carried out. Among the two extracts tested by MTT assay, the ethanol extract showed good IC50 value followed by water extract at a concentration of 3.0 and 10.8 µg/ml

against CEMss cells. The results were recorded in Table 3 and Figure 1. Morphological and ultra-morphological assessments of apoptosis produced by the active ethanol extract on CEMss cell line were identified by phase contrast, fluorescence and scanning electron microscopes. The mode of cell death occurred by ethanol extract was found to be through apoptosis and arresting the cell cycle at G0/G1 phase. The current study reveals that the apoptosis was occurred via mitochondrial pathways.

Table 3 Effect of different concentrations of ethanol and water extracts of *R. sambucifolia* on the viability of Human T4-lymphoblastoid cell line for 48 h.

| Concentration (µg/ml) | % Viability | |
|---|---|---|
| | Ethanolic extract | Water extract |
| 1 | 100 | 100 |
| 1.5 | 82 | 91 |
| 3 | 50 | 74 |
| 6 | 36 | 65 |
| 12 | 24 | 41 |
| 24 | 24 | 30 |

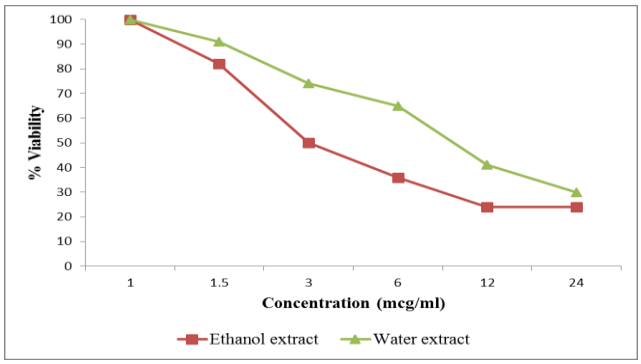
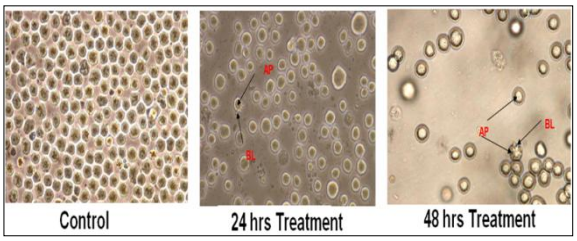



Figure 1 Effect of different concentrations of ethanol and water extracts of *R. sambucifolia* on the viability of Human T4-lymphoblastoid cell line for 48 h.

Normal inverted microscope studies was carried out for the ethanolic and water extracts to observe the morphological changes occurs in the cells, which was well compared with untreated cells. Figure 2 showed several morphological changes of the extract treated cells compared with untreated cells at 48 h post treatment under 400 x magnifications. Treated CEMss cells showed the blebbing of the cell membrane and more promising growth incubation and shrinking of cells. When the cell treated with both the extracts for 48 h where stain with AO and PI and examined under the fluorescence microscope. The characteristic of the apoptotic features such as cell shrinkage, membrane blebbing, and late apoptosis were seen (Figure 3). Morphological analysis of treated CEMss cells was carried out to gain inside into morphological caused by ethanolic extract of *R. sambucifolia*.

Both extra and intracellular structure analysis was carried out by SEM and TEM respectively. Interpretation of SEM electromicrographs showed distinctive morphological changes corresponding to a typical cellular surface morphology of apoptotic, cells shrinkage, and blebbing. The intra morphological features observed by TEM demonstrated clear morphological changes in the nucleus with the formation of sharply, uniformly and finely granular mass which marginated against the nuclear envelope (Figure 4). These apoptosis affects were found to be time correlated




phenomena and this was noticed when considering the number of blebs formation as an indicator of cell death via apoptosis.

Figure 2 The morphological changes of CEMss cells after 48 h treatment

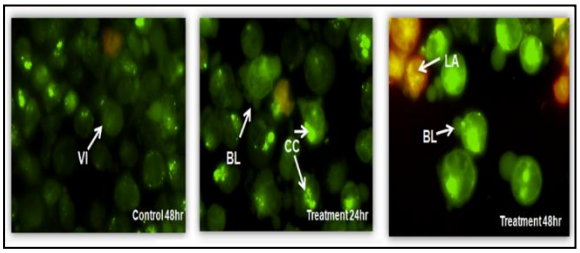



Figure 3 Fluorescence microscopic analysis of CEMss treated for 48 h with ethanolic extract of *Rodgersia sambucifolia*

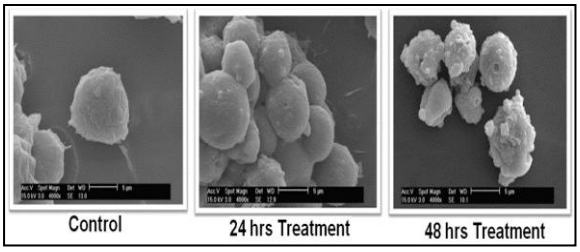



Figure 4 Morphologic changes during apoptosis by Scanning Electron Microscope

## IV. DISCUSSION

The water extract of *R. sambucifolia* shows maximum percentage of yield which is shown in Table 1. The phytochemical analysis revealed the presence of alkaloids, carbohydrates and glycosides, tannins and phenolic compounds and flavonoids and the absence of proteins and amino acids, sterols, fixed oils and fats in ethanol and water extracts. The TLC profile of ethanol and water extracts revealed that the ethanolic extract of *R. sambucifolia* showed 2 spots at the mobile phase of butanol:aceticacid:water and phenol:water respectively. And the water extract shows 3 spots at the mobile phase of butanol:acetic acid:water and phenol:water respectively. In HCl:acetic acid:water showed 2 spots at a $R_f$ value of 0.19 and 0.60.

Morphological changes found in this study by normal inverted microscope showed the morphological signs of apoptosis which was very clear especially the cellular blebbing and loss of shape of cytoplasm. By fluorescence

microscope, the characteristics such as cell shrinkage, membrane blebbing, and late apoptosis were observed. However, the extract of *R. sambucifolia* shown anticancer effect on CEMss leukemic cell line for the first time.

The ethanol and water extracts shows the presence of alkaloids in the qualitative phytochemical evaluation. Mostly alkaloids will be responsible for the cytotoxic effect of *R. sambucifolia*. To carry out the isolation, structure elucidation and the exact mechanism for anticancer property is necessary before declaring *R. sambucifolia* as an anticancer agent.

## V. CONCLUSION

In conclusion, the ethanolic and water extracts of R. sambucifolia inhibit the cell proliferation in-vitro on CEMss human T4-lymphoblastoid cell lines. In addition to this, the possible mechanism action of this plant also were tried to establish. Future work is required in order to fractionate the active constituent and to evaluate the anticancer property of the isolated compound. Such a study in the discovery of anticancer agent is the need of this hour.

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