INTRODUCTION

Paris polyphylla LINN-HS 703.3 (herbarium number) (Herb Smith) SM.avr.yunnanensis, Chinese (chonglou) Hindi name (Doodh Buch) and in Nepalese (Satua) a perennial herb originates from the Himalayas. It usually grow about 10-100 cm tall from a rhizome 1-2.5 cm thick. The rhizomes of Paris polyphylla have been used as anti-helmintic and vermifuge in folk communities of Nepal1. The powdered roots of this plant are used as ethnopediatrics for diarrhoea in Garwal Himalaya, Uttarakhand, India2. It is one of the medicinally important plants in traditional Chinese medicine. Rhizomes of Paris polyphylla have saponin glycosides. Diosgenyl and with different sugar chains at 3-hydroxy group are the major saponin glycosides present in the rhizome. These compounds have been used as hemostatic agents and promoters for shrinkage of uterus in clinics. They also exhibit antibiotic and antitumor activity3,4,5. Medicinal plants have been in use from time immemorial and their utility has been increasing day by day in the present world. Naturally obtained compounds are considered safer and easily biodegradable than synthetic compounds and the problem of drug resistance observed in synthetic drugs is also reduced6. Plants represent a source of leads for many pharmaceutical compounds and the phytochemical compounds and secondary metabolites present in the plants have been in treating a number of human ailments. Drugs obtained from medicinal plants comprise 25% of total drugs in developed countries and about 80% in developing countries6. Lung cancer has been regarded as a leading cause of cancer-related mortality throughout the World. Its occurrence and development are associated with a variety of factors, including oxidative stress, apoptosis, immune factors disorders, dysfunction of lung epithelial cells, inflammation, etc. Phytochemical study showed that its main components, steroidal saponins displayed a potential cytotoxicity against various tumor cells, such as CCRF leukemia cells, ECA109 esophageal cancer cells, CaEs-17 cells, human promyelocytic leukemia HL-60 cells, human liver carcinoma HepG-2 cells, human gastric cancer BGC-823 cells, human colon adenocarcinoma LoVo cells and SW-116 cells7,8.

MATERIALS AND METHODS

Preparation of herbal extract

The rhizomes of the plant were dried in shade for about 3 weeks and ground using a mixer to a coarse powder. Using a soxhlet extraction method, the powder of dried rhizomes were processed with petroleum ether (40-50°C) for 18 h in order to remove fat and unwanted components. The treated powder was further processed with hydroalcoholic solution (25:75) by using same extraction process for 18 h. The extract was concentrated by evaporating the solvent using a water bath maintaining at 60-800C at ambient conditions to get a crude hydroalcoholic extract devoid of solvents.

Identification of Chemical Components

Steroidal saponins were the main compounds of Paris polyphylla and they have been confirmed as contributors to the inhibition of tumor growth9. After being extracted with hydroalcoholic method, these main steroidal saponin compounds were analyzed by NMR and identified by high performance liquid chromatography-diode array detection (HPLC-DAD) and liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS).

MTT Assay

Cytotoxicity of the hydroalcoholic rhizome extract of Paris polyphylla was studied using A549 lung cancer cell line. The MTT assay was performed here as it allows assessing the viability and the proliferation of cells and thus giving an insight into cytotoxic potential of drugs. The MTT assay measures the activity of enzymes that reduce MTT to formazon dyes, giving a purple color. The amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control
cells, the effectiveness of the agent in causing death can be deduced through the production of a dose response curve. DMEM/F-12 Media and MTT were purchased from ATCC and Sigma respectively. Doxorubicin Hydrochloride was used as a reference drug along with the Paris polyphylla extract. Data Analysis was performed using GraphPad Prism software.

**MTT Assay protocol**

**DAY1**

**Step 1**
Plate 7000 cells in 100 ul media per well in a 96 well cell culture plate.

**Step 2**
Incubate (37°C, 5 % CO₂) overnight to allow the cells to attach to the wells.

**DAY2**

**Step 3**
Prepare serial dilution of the Doxorubicin and hydroalcoholic extract in DMEM/F-12

**Step 4**
Add the respective concentrations of the drugs to the assigned wells as per the plate plan with cells. Place on a shaking incubator @ 150RPM for 5 minutes, to thoroughly mix the samples into the media.

**Step 5**
Incubate (37°C, 5 % CO₂) for 72 h to allow the drug to take effect.

**DAY5**

**Step 6**
Aspirate and add 100 ul media and 20 ul MTT solutions to each well. Place on a shaking table, 150 rpm for 5 minutes, to thoroughly mix the MTT into the media.

**Step 7**
Incubate (37°C, 5 % CO₂) for 1-5 hours to allow the MTT to be metabolized.

**Step 8**
Remove the media from the plate by aspiration.

**Step 9**
Add 100 ul DMSO to each well. Place on a shaker at 150 rpm for 5 minutes, to thoroughly mix the formazan into the solvent.

**Step 10**
Read optical density at 570 nM. Optical density should be directly correlated with cell quantity.

**Plate plan**
In a 96 well plate, the treatment scheme mentioned in Table 1.

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<td>500 ug/ml</td>
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Figure 1 1H1 NMR of hydroalcoholic extract for Diosgenyl saponins
Figure 2 HPLC chromatogram (A) and LC-ESI-MS/MS spectra
Figure 3 LCMS spectra of hydroalcoholic extract compounds

Figure 4 EC50 of extract in µg/ml

Figure 5 EC50 of Doxorubicin in µg/ml
RESULT

Spectral Analysis

NMR analysis of the hydroalcoholic extract has shown major structure f component Diosgenyl saponin as shown in Figure 1 and hydrogen shifts in 1H NMR DMSO-D6: 0.78–0.79 (m, 6H, CH3-18, CH3-27), 0.96–1.00 (m, 6H, CH3-21, CH3-19), 1.28 (d, 3H, J = 6.0 Hz, CH3 in rhamnose), 1.34, 1.35 (s each, 3H each, O), 3.37 (t, 1H, H-O), 3.43 (t, 1H, J = 7.8; 8.4 Hz, H-3”), 3.47 (m, 1H, H-26b), 3.50 (m, 1H, H-3”), 3.75 (dd, 1H, J = 4.2; 8.4 Hz, H-5’a), 3.83 (t, 1H, J = 7.2; 7.8 Hz, H-2’), 3.99 (m, 1H, H-5”), 4.05 (m, 1H, H-4”), 4.07 (m, 1H, H-3”), 4.15 (m, 1H, H-3’), 4.82 (m, 1H, H-4’), 5.03 (s, 1H, H-1a), 5.04 (s, 1H, H-1b). Hydroalcoholic extract analysed for steroidal saponin and identified by high performance liquid chromatography-diode array detection (HPLC-DAD) and liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). As shown in Figure 2 HPLC Dad chromatogram has shown many components and as shown in Figure 3. Maximum ion strength has gone upto 1046 m/z for pennogenyl saponins and 796 m/z for Diosgenyl saponins.

MTT assay for cell viability

More than 97 % increment in cell killing at a concentration of 500 µg/ml recorded in the cell line. The EC50 for the extract was calculated to be 52.34 µg/ml see Figure 4 and at the concentration of 1.8559 µg/ml, doxorubicin exhibited approximately 98 % killing of the cells. The EC50 for the Doxorubicin was calculated to be 0.579 µg/ml see Figure 5. From the study it is concluded the hydroalcoholic rhizome extracts Paris polyphylla has potential to exhibit anticancer activity.

REFERENCES


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