Anticancer activity of *Artemisia pallens*

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Abstract

Current study focusses about the anticancer activity of *Artemisia pallens Wall. ex Dc* Water, methanol and chloroform extracts were analysed for their potent anticancer activity. The chloroform extract seems to be very good when compared to the water and methanol extracts. These may be attributed to the compounds present in A.pallens. The anticancer property may be due to the presence of many natural compounds.

Key words: A.pallens, anticancer, MTT assay, HeLa cells

Introduction

Ethnomedicine is one of the most promising field which cure various human ailments. Phytoconstituents contains specific characteristic values with high intensity towards the electrostatic ionic molecules in the living cells (Paikara and Pandey 2018). *Artemisia pallens* Wall. ex Dc is an aromatic xerophytic herb belongs to the Asteraceae family. *A. pallens* are widely used to treat cancer and diabetes. The anticancer activity of the *A. pallens* has not been explored (Renuka *et al.,* 2017).

Cancer is defined as a disease where an abnormal group of cells grow uncontrollably ignoring the cell divisions. Normal cells follow cell signalling, divide, differentiate into another and die (Momna, 2013). Cancer is said to be the second leading cause of death (WHO, 2022). In developing and underdeveloped countries, cancer treatments are really expensive which cannot be afford by all the people. Various allopathic medicines and treatments for cancer resulted various side effects and resurrection (Palombo and Semple 2001). Many pharmaceutical and research industries looking for the bioactive product which is cost effective, target-oriented with less side effects (Suresh *et al.*, 2007).

Nowadays, chemotherapeutic drug development from the active compounds isolated from native medicinal plant extracts are gained a much attention (Uniyal *et al.*, 2006). Generally, tumor cells have the ability to resist the administered antitumor drugs by enhancing genetic mutation and enormous mitosis cell division (Liu *et al.*, 2018). Cervical cancer is the fourth most frequent cancer in women with an estimated 570,000 new cases in 2018 representing 6.6% of all female cancers (WHO, 2018). Cancer of the cervix is one of the common form of cancer after breast cancer. (Geetha and Santhy 2013). HeLa cells are used to detect the cervical cancer. In India, cervical cancer has been the most important cancer in women over past two decades. (Nandakumar *et al.*, 2009). The scope of the present study is to evaluate the anticancer property of *A. pallens*.

2. Materials and Methods

2.1 Plant Material

A.pallens was procured from the flower markets of Coimbatore, Tamil Nadu, India. The plant was identified and authenticated by the Botanical Survey of India, Coimbatore, Tamil Nadu, India.

2.2 Extraction Procedure

The leaves of the plants were shredded with the help of scissors. The water, methanol and chloroform extracts were prepared by extracting with a shaker with an RPM of 70 and kept at a temperature of 40°C. The extracts were stored in a sealed container for further use.

2.3 Media Preparation

0.0037 g of sodium carbonate and 0.0045 g of glucose was made up to 1000ml. To this 0.097 g of Dulbecco's Modified Eagle Medium (DMEM) was added. The DMEM medium was prepared in the above mentioned procedure and 0.5 g of Fetal Bovine Serum (FBS) was dissolved in 100 ml of distilled water. The FBS medium was prepared by the above mentioned procedure.

2.4 Cell Lines and Culture Medium

The human cervical cancer cell line (HeLa) was obtained from National Center for Cell Sciences (NCS), Pune, Maharashtra. The cells were maintained in a CO_2 incubator at a pH of 7, temperature of 37.5 °C and a relative humidity of 80%. The culture medium was also incubated for about 24-72 hours.

2.5 Cell Treatment Procedure

After incubation for 24-72 hours, the cell along with the culture medium were taken out. The medium along with the cells were fed into a 96-well plates. The 1st well contained Dimethyl Sulfoxide (DMSO). This constituted the blank. 10 µl, 20 µl and 30 µl of water, methanol and chloroform extracts were taken in nine tubes separately. DMSO and the cell lines were kept in the 2nd well. The 3rd, 4th and 5th well contained the 10, 20, 30 µl of water extract along with the cell lines. The 6th, 7th and 8th well contained the 10, 20, 30 µl of methanol extract along with the cell lines. The 9th, 10th, and 11th well contained the 10, 20, 30 µl of chloroform extract along with the cell lines and the 12th well had the cell lines in it. These were then incubated in the CO_2 for about 24 hours.

2.6 MTT assay

The MTT assay was carried out according to (Geetha *et al., 2013)*. After 24 hours incubation the contents in the 96-well plate was washed with DMSO followed by Trypsin. After washing with DMSO and Trypsin 10 µl of MTT dye was added to all the wells. It was later mixed well and incubated in the CO_2 incubator for about 2-4 hours. After 2-4 hours incubation, the plates were measured in an ELISA reader with an absorbance of 570 nm. The % cell inhibition was determined by the formula: = 100 -Absolute (Sample) / Absolute (Control) x 100.

RESULTS

The cytotoxic effects of water extract of *Artemisia Pallens* at 10, 20 and 30 µg/ml was 11.11µg/ml, 24.39 µg/ml, and 45.45 µg/ml, methanol extract at 10, 20, 30 µg/ml was 13.89µg/ml, 29.41 µg/ml and 71.43 µg/ml and the chloroform extract at 10, 20, 30 µg/ml was 9.09 µg/ml, 23.26 µg/ml and 44.12 µg/ml.

DISCUSSION

The water extract of *A.annua* without silicate infusion was higher than 6.25 µg/ml, with silicate infusion was higher than 285.71 µg/ml and pure artemisin was higher than 55.56 µg/ml. The above said results suggest that *A.annua* with or without silica infusion showed higher level of cytotoxic activity than *A.pallens*. These results suggest that Artemisin which is a derivative of *A. annua* could be used to prepare anticancer medicines. (Cristina et. Al., 2016)

The MTT assay of *Artemisia ciniformis* was found to be 19.64 µg/ml. This showed that the cytotoxic assay of *A.pallens* seems to be better. This may be due to its natural compounds responsible for this activity. (Taherkhani, 2016)

Fig-1 Photo of raw material artemisia pallens

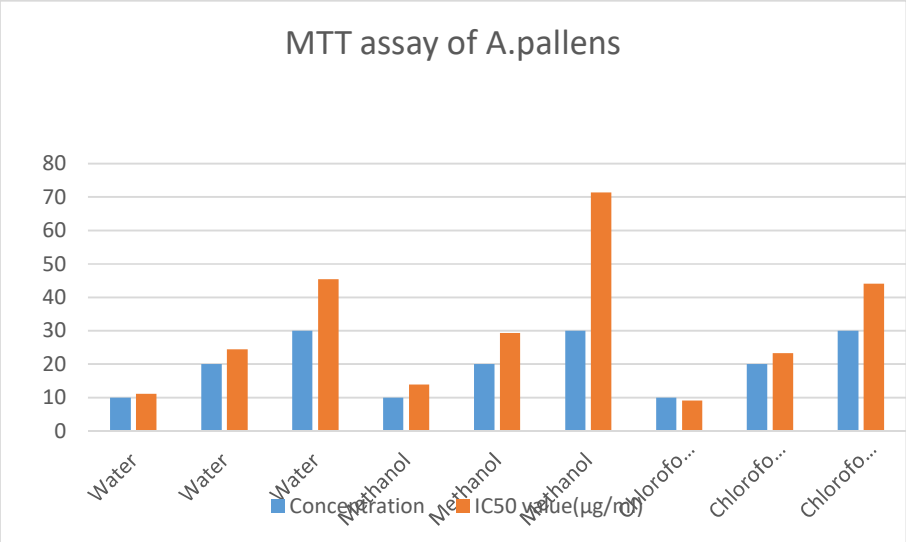

Fig-2 MTT assay of *A.pallens*

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K. Sai Pavithra

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