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Research Article

ANTICANCER ACTIVITY AND DNA FRAGMENTATION ACTIVITY OF *A. PALLENS* EXTRACTS

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ABSTRACT

Objective*: The present study is to evaluate the anticancer activity of the leaf extracts of A. Pallens.* ***Methods****: Water, methanol and chloroform extracts were analyzed for their potent anticancer activity.* ***Results****: 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.* ***Conclusion****: The anticancer property may be due to the presence of many natural compounds.*

KEYWORDS: *A. Pallens, Anticancer, MTT assay, HeLa cells.*

INTRODUCTION

Artemisia pallens Wall. ex Dc is an aromatic xerophytic herb belonging to *Asteraceae* family. They are native of India. [1] *A. Pallens* has been used to treat cancer and diabetes. The anticancer activity of this plant has not been explored [2].Cancer is defined as a disease where an abnormal group of cells grow uncontrollably ignoring the cell divisions. Normal cells follow cell signaling, divide, differentiate into another and die [3]. Cancer is said to be the second leading cause of death. [4] There are many cell lines or cell cultures used to distinguish various types of cancer. Cancer of the cervix is one of the common form of cancer after breast cancer [5]. HeLa cells are used to detect the cervical cancer. Cancer of the cervix has been the most important cancer in women in India, over past two decades. [6] 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. [7] The scope of the present study is to evaluate the anticancer property of *A. Pallens.*

MATERIALS AND METHODS

Plant Material and Extract Preparation:

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

Cytotoxicity studies:

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.

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.

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 second 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.

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MTT assay:

The MTT assay was carried out according to [5]. 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.

DNA Nicking assay:

The DNA nicking assay was performed using the standard method according to [8] using pBR322 plasmid DNA. Plasmid DNA (3µl) was added to Fenton's reagent (30 mM H_2O_2, 50 mM Ascorbic acid, and 80 mM $FeCl_3$) having 10 µl of water, methanol and chloroform reagents and the final volume of the mixture was brought up to 25 µl using double distilled water. The reaction mixtures were allowed to incubate for 40 min at 37°C. After 30 min incubation, Bromophenol blue dye (0.25% in 50% glycerol) was added. The reaction mixtures (20 µL) were loaded on 0.8% agarose gel (prepared by dissolving 0.4 g of agarose in 50 mL of 1 × TAE Buffer) and electrophoresis was carried out at 70 V for 1 hour followed by Ethidium bromide staining.

RESULTS

The cytotoxic effects of water extract of *Artemisia Pallens* at 10, 20 and 30 µg/ml was 11.11µg/ml, 24.39µg/ml, and 45.45µg/ml, methanol extract at 10, 20, 30µg/ml was 13.89µg/ml, 29.41µg/ml and 71.43µg/ml and the chloroform extract at 10, 20, 30µg/ml was 9.09µg/ml, 23.26µg/ml and 44.12µg/ml. DNA fragmentation of *Artemisia pallens* showed no fragmentation.

DISCUSSION

In the present study, the MTT assay of *A. Pallens* of water extract was 45.45µg/ml, methanol extract was 71.43 and chloroform extract was 44.12µg/ml.

With respect to [9], the MTT assay of 20 µl of dihydroartemisin, a derivative of *A.annua* was found out to be 66.67µg/ml. This suggests that *A. Pallens* has very good cytotoxic activity.

In the present study, the MTT assay of *A. Pallens* of water extract was 45.45µg/ml, methanol extract was 71.43 and chloroform extract was 44.12µg/ml.

According to [10], the water extract of *A.annua* without silicate infusion was higher than 6.25µg/ml, with silicate infusion was higher than 285.71µg/ml and pure artemisin was higher than 55.56µg/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.

In the present study, the MTT assay of *A. Pallens* of water extract was 45.45µg/ml, methanol extract was 71.43 and chloroform extract was 44.12µg/ml.

According to [11], the sesquiterpene lactone obtained from *Artemisia macrocephala,* the 1000µg/ml showed 617.38µg/ml, 500µg/ml showed 390.62µg/ml, 250µg/ml showed 235.84µg/ml, 125µg/ml showed 168.91µg/ml and 62.5µg/ml showed 130.2µg/ml. These suggest that *Artemisia macrocephala* shows more cytotoxic effect when compared to the MTT assay of *A.pallens.* This suggests that *Artemisia macrocephala* seems to have very good anticancer activity.

In the present study, the MTT assay of *A. Pallens* of water extract was 45.45µg/ml, methanol extract was 71.43 and chloroform extract was 44.12µg/ml.

With respect to [12], the MTT assay of *Artemisia ciniformis* was found to be 19.64µg/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.

In the present study, the MTT assay of *A. Pallens* of water extract was 45.45µg/ml, methanol extract was 71.43 and chloroform extract was 44.12µg/ml.

The [13] shows that the ethyl acetate extract of *Artemisia nilagirica* was 51.67µg/ml and water extract was 92.25µg/ml. The above results suggest that the water extract of *Artemisia nilagirica* shows good results when compared to all the results of *A. Pallens* and the ethyl acetate results are low when compared to *A.pallens*.

In the present study, the MTT assay of *A. Pallens* of water extract was 45.45µg/ml, methanol extract was 71.43 and chloroform extract was 44.12µg/ml.

According to [14], the MTT assay of fragment 9 of *Artemisia sieberi* was found to be 23.6µg/ml, fragment 10 was 18.3µg/ml, fragment 11 was 63.2µg/ml, fragment 12 was 12.5µg/ml, fragment 13 was 8.1µg/ml and fragment 14 was 13.8. These values when compared to the values obtained from *A.siberi* seem to be lower than the MTT assay values of *A.pallens.*

In the present study, the MTT assay of *A. Pallens* of water extract was 45.45µg/ml, methanol extract was 71.43 and chloroform extract was 44.12µg/ml.

According to [15], the MTT assay of the methanol extract of *A.annua* was 360µg/ml, ethyl acetate was 1675µg/ml, dichloromethane was 388µg/ml and n-hexane was 168µg/ml, methanol extract of *A.biennis* was 288µg/ml, ethyl acetate was 1050, dichloromethane was 74µg/ml and n-hexane was 301, methanol extract of *A.ciniformis* was 130µg/ml, ethyl acetate was 73µg/ml, dichloromethane was 97µg/ml and n-hexane was 300µg/ml, the methanol extract of *A.diffusa* was greater than 2000µg/ml, ethyl acetate was 154µg/ml, dichloromethane was 71µg/ml and n-hexane was 205µg/ml, the methanol extract of *A.persica* was greater than 2000µg/ml, ethyl acetate was 248µg/ml, dichloromethane was 219µg/ml and n-hexane was 138µg/ml, the methanol extract of *A.santolina* was greater than 2000µg/ml, ethyl acetate was 221µg/ml, dichloromethane was 538µg/ml and n-hexane was 357, the methanol extract of *A.vulgaris* was greater than 2000µg/ml, ethyl acetate was 387µg/ml, dichloromethane was 351µg/ml and n-hexane was 160µg/ml. These results suggest that the *A.biennis, A.ciniformis, A.diffusa, A.persica, A.santolina* and *A.vulgaris* showed best results than the *A.pallens*.

In the present study, the MTT assay of *A. Pallens* of water extract was 45.45µg/ml, methanol extract was 71.43 and chloroform extract was 44.12µg/ml.

With respect to [16], the MTT assay of the *Artemisia asiatica* was Artecanin which is a sesquiterpene lactone obtained from *A.asiatica* was found to be IC50 21.76µg/ml, 3β-Chloro-4α,10α-dihydroxy-1α,2α-epoxy-5α,7αHguai-11(13)-en-12,6α-olide which is also a sesquiterpene lactone was 11.46µg/ml, Hispidulin which is a flavonoid was 5.68µg/ml, Iso-seco-tanapartholide 3-O-methyl ester a sesquiterpene lactone was found to be 12.04µg/ml, Jaceosidin a flavonoid was 23.58µg/ml, Cirsilineol a flavonoid was 15.24µg/ml, 5,7,4′,5′-Tetrahydroxy-6,3′-dimethoxyflavone a flavonoid was 2.25µg/ml and 6-Methoxytricin a flavonoid was 24.19µg/ml. The values obtained from the MTT assay of *A.asiatica* seems to be lower than the values obtained from *A.pallens.* These suggest that *A. Pallens* due to its potent cytotoxic activity can be used to prepare anticancer medicines.

In the present study, the MTT assay of *A. Pallens* of water extract was 45.45µg/ml, methanol extract was 71.43 and chloroform extract was 44.12µg/ml.

According to [17], the MTT assay of the ethanolic extract of *Artemisia afra* was 31.88µg/ml. The values obtained from the MTT assay of *A.afra* seems to be lower than the values obtained from *A.pallens.* These suggest that *A. Pallens* may possess anticancer activity.

In the present study, the MTT assay of *A. Pallens* of water extract was 45.45µg/ml, methanol extract was 71.43 and chloroform extract was 44.12µg/ml.

According to [18], the flower extract of *Artemisia asiatica* was found to be 5.99µg/ml and the folium extract was 10.42µg/ml, the flower extract of *Artemisia japonica* was 9.72µg/ml and the folium extract was 6.89. This suggests that both the extracts of *Artemisia asiatica and A. Japonica* seems to be lower than all the extracts of *A. Pallens.* These may be due to the elevated level of cytotoxic activity in *A. Pallens.*

In the present study, the MTT assay of *A. Pallens* of water extract was 45.45µg/ml, methanol extract was 71.43 and chloroform extract was 44.12µg/ml.

With respect to [19], the cytotoxic effect of the ethanolic extract of *Artemisia afra* was found to be 30µg/ml. This value indicates that *A. Pallens* has very good cytotoxic activity.

In the present study, the MTT assay of *A. Pallens* of water extract was 45.45µg/ml, methanol extract was 71.43 and chloroform extract was 44.12µg/ml.

The [20] states that the MTT assay of the sample 1 of methanol of *Artemisia khorasanica* was 76µg/ml, ethyl acetate was 63µg/ml, dichloromethane was 69µg/ml and hexan was 563µg/ml, for the sample 2 the methanol extract was 183µg/ml, ethyl acetate was 153µg/ml, dichloromethane was 66µg/ml and hexan was 2993µg/ml and for sample 3 the methanol was 465µg/ml, ethyl acetate was 91µg/ml, dichloromethane was 77µg/ml and hexan was 312µg/ml. These above findings shows that *A.khorasanica* has very good cytotoxic activity when compared to *A.pallens.*

In the present study, the MTT assay of *A. Pallens* of water extract was 45.45µg/ml, methanol extract was 71.43 and chloroform extract was 44.12µg/ml.

According to [21], the cytotoxicity of Artesunate which is a derivative of *Artemisia annua* was 35.4µg/ml. This suggests that *A. Pallens* has good cytotoxic activity. This can be also be used to prepare anticancer medicines.

In the present study, the MTT assay of *A. Pallens* of water extract was 45.45µg/ml, methanol extract was 71.43 and chloroform extract was 44.12µg/ml.

The [22] shows the MTT assay of ethanol extract of *Artemisia afra* to be 8.15µg/ml. This values suggests that *A. Pallens* has very good cytotoxic activity.

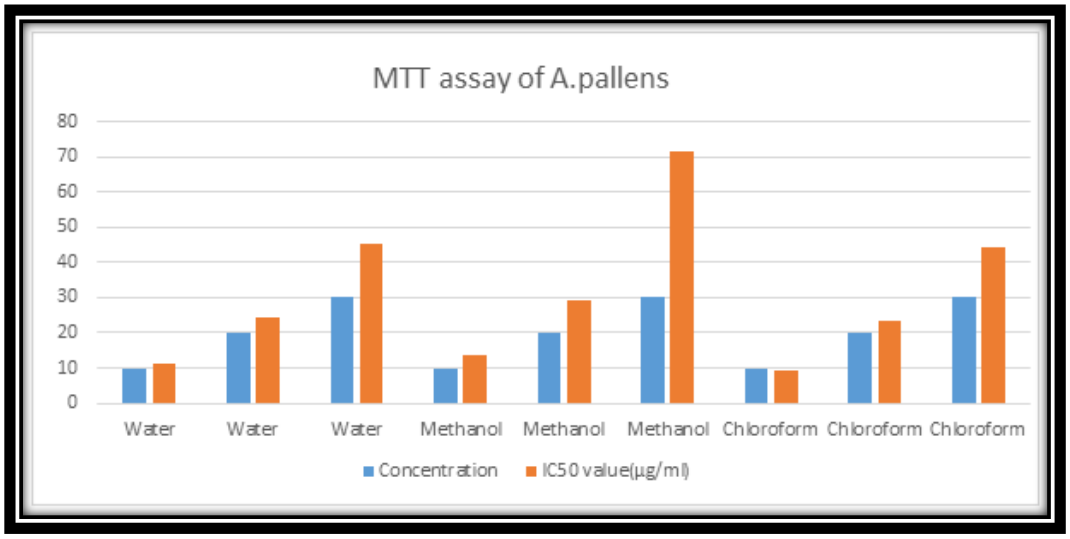

Fig. 1: MTT assay of *A.pallens*

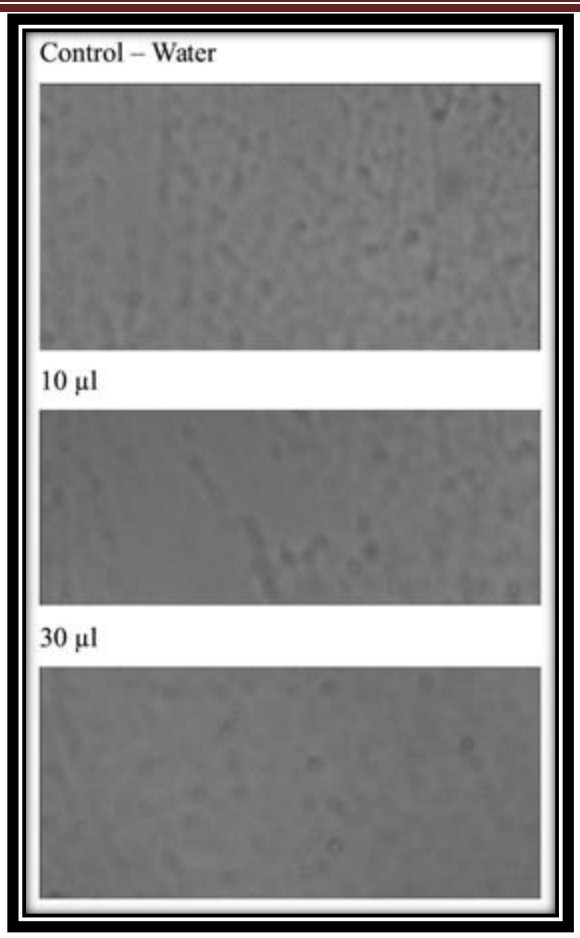

Fig. 2: MTT assay of water

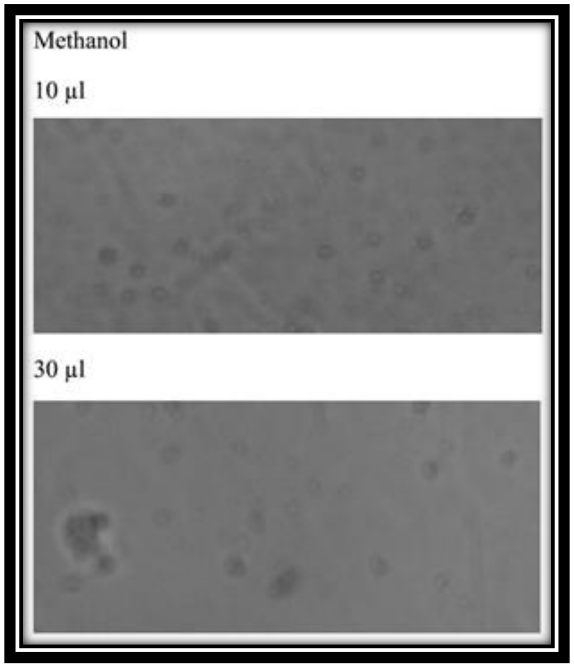

Fig. 3: MTT assay of methanol

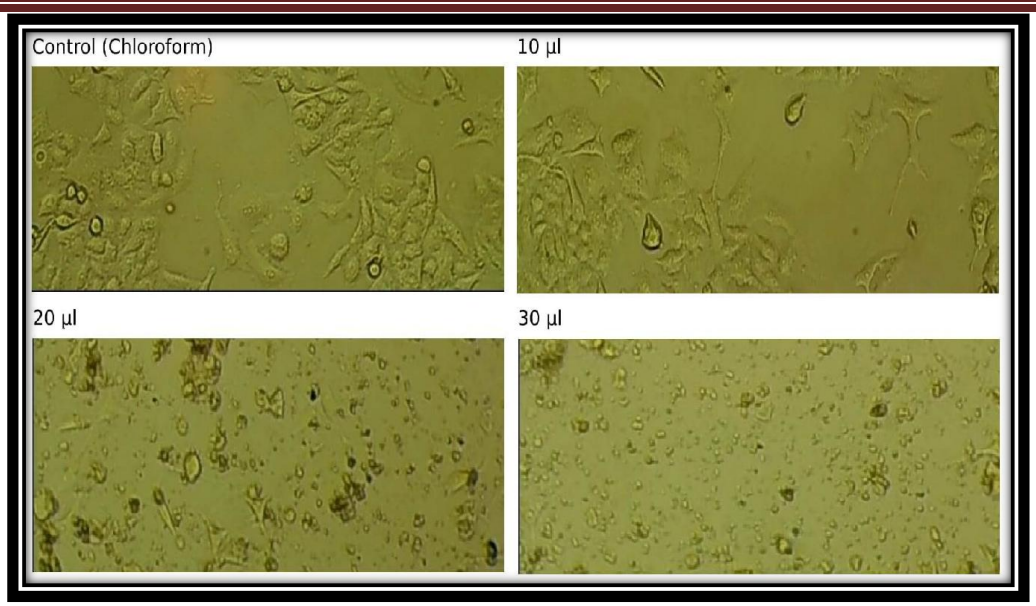

Fig. 4: MTT assay of chloroform

Fig. 5: DNA fragmentation

CONCLUSION

A*rtemisia pallens* showed fair cytotoxic effects towards its chloroform extracts than the water and methanol extracts.

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