ANTICANCER ACTIVITY OF ANDROGRAPHIS PANICULATA LEAVES EXTRACT AGAINST NEUROBLASTIMA (IMR-32) AND HUMAN COLON (HT-29) CANCER CELL LINE

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ABSTRACT
Cancer is one of the most leading cause of death worldwide. Plants are used to cure various diseases which are known to possess anticancer activities against different human cancer cell lines. In this report, we studied the invitro anticancer properties of Andrographis paniculata leaves against neuroblastima (IMR-32) and human colon (HT-29) cancer cell line. The leaves were shade dried and extracted with water, ethanol and acetone solvents. Anticancer property of A. paniculata leaf extract was analyzed by Spectrophotometric MTT assay method. The results were found that ethanol extract showed nearly 50% i.e. inhibition concentration (IC₅₀) for IMR-32 and HT-29 cell lines at 200 μg/ml, where other extracts display 50% inhibition at 250 μg/ml concentration for HT-29 cell lines. Anticancer activity of water, ethanol and acetone extracts of A. paniculata leaves against HT-29 cancer cell lines shows 50% inhibition at 200 μg/ml concentration. The significant difference is statistically analyzed as p<0.01 for ethanol extract and acetone extracts. From the analysis we found that extracts of A. paniculata shows excellent anticancer activities against different cancer cell lines, it is alternatives medicines for cancer would replace side effect causing chemotherapeutic agent.
KEYWORDS: Andrographis paniculata, invitro, MTT assay, human cancer cell lines, anticancer.

INTRODUCTION
Cancer is one of the most common leading causes of mortality worldwide. Cancer is an uncontrolled growth of cells resulting in lack of differentiation and ability to invade local tissues and metastasis which are proliferate individually throughout the body. During metastasis, cancer cells enter the blood stream and are carried to distant parts of the body where they form other similar growths (Jemal et al., 2008). Synthetic drugs are available for the treatment of cancer but they are not free from adverse effects. Chemotherapy and radiation therapy are major clinical treatment used for the control of early stages of tumor but these methods has serious side effects (Hogland 1982). However, alternative and complementary methods were need to improve the treatment of diseases like cancer (Thanangkuland Chaichantipyuth, 1985). Nature has provide human a variety of useful sources mainly plants for discovery and development of drugs against dreadful diseases (Joselin and Jeeva2014). Traditional herbs as an effective system of treatment of cancer and many diseases (Sundaram et al., 2011). Drugs from medicinal plants are found to be comparatively less toxic and side effects (Farnsworth, 1988).

Medicinal plant Andrographis paniculata belongs to the Acanthaceae family and commonly known as the King of bitters. Roots and leaves from this herbaceous plant was used for the treatment of respiratory infections, sore, throat and other chronic and infectious diseases. Its native is India and Srilanka and usually it cultivated in Southern Asia. Leaves has many phytochemical constituents like phenols, tannins, alkaloids, saponins flavonoids and reducing sugars. These phytochemicals actively involved in the medicinal uses for treating various diseases. This plant has many medicinal activity such as antimicrobial (Zaidan, et al 2005), anti-inflammatory (Abu-Ghefreh et al 2009), anti-oxidant (Trivedi and Rawal, 2001), anti-allergic, hepatoprotective activity (Vetriselvan et al 2011), and nephroprotective activity. The plant extract also exhibits antityphoid, antifungal, antimalarial anti thrombogenic, anti-snake venom and antipyreticproperties. Besides this it is also use as an immunostimulant agent. In the present report, we report invitro anticancer activities of different solvent derived extracts of A. paniculata leaves against different human cancer cell lines are neuroblastima (IMR-32) and human colon (HT-29)cells.
MATERIALS AND METHODS

Chemicals
Analytical graded3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethylsulfoxide (DMSO), doxorubicin and other chemicals were purchased from Himedia laboratories private limited, Mumbai. The medicinal plant A. paniculata plant was collected from Vandhavasi, South India.

Preparation of plant extracts
The leaves of *Andrographis paniculata* were collected and shade dried for 3-5 days. The shade dried leaves were subjected to maceration to get coarse powder which was then used for extraction with water, ethanol and acetone. Water extract was prepared by immersing 100 g dried leaf powder into 200 ml double distilled for 24 hours. 100g of dry powder was loosely packed in the thimble of soxhlet apparatus and extracted with 80 % ethanol at 55°C for 24 hours. Acetone extract was prepared by adding dried powder with the solvent 80% acetone. The extracts were left to evaporate in the air at room temperature yielding a concentrated water, ethanol and acetone extract which was used for the anticancer studies.

Anticancer activity against neuroblastima (IMR-32) and human colon (HT-29) cancer cell line
Cells were tested for viability by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Cells were plated separately in 96 well plates at a concentration of $1 \times 10^4$ cells/well and exposed to serially dilutions of aqueous, ethanol and acetone extract of A. paniculata for 24 h. then the cells were changed to serum free medium containing MTT and incubated for 4 hours in CO$_2$ incubator at 37°C. The spectrophotometric MTT assay assessed based on the ability of living cells to reduce soluble yellow MTT into insoluble purple formazan. Formazan crystals were dissolved using DMSO and the optical density was measured at 570 nm. The 50 % of inhibitory concentration value (IC$_{50}$) of the extracts was identified for normal untreated cell line. Commercial anticancer drug Doxorubicin was used as a positive control. The assay was performed in triplicate for each extracts.

$$\% \text{ Cell viability} = 1 - \frac{\text{Absorbance for treated cells}}{\text{Absorbance for control cells}} \times 100$$
Statistical analysis

Quantitatively obtained values were characterized by regression analysis used to compute the 50 % inhibition concentration (IC$_{50}$) in cell viability. Results were expressed as the mean ± SD of values obtained in triplicate from three independent experiments. Statistical differences between correlated samples were noted to be significantly different where $p < 0.05$ using one-way analysis of variances (ANOVA).

RESULTS

Anticancer activity of plant extracts

*In vitro* assay of anticancer activity of aqueous, ethanol and acetone extract of *A. paniculata* leaves against IMR-32 and HT-29 cancer cell lines at different concentrations was evaluated by MTT assay (Table 1 and 2). MTT assay is based on the metabolic reduction of MTT into formazan crystals on treatment with cancer cell lines. The inhibitory activities of these extracts were compared with the standard drug doxorubicin for IMR-32 and HT-29 cancer cell lines. The cancer cell viability percentage were found to be at different concentration of extracts (Table 1 and 2). Anticancer activity at the different concentrations of 50 µg, 100 µg, 150 µg, 200 µg, 250 µg and 300 µg/ml showed effective inhibition against cancer cell lines. All the extracts were active against IMR-32 and HT-29 cancer cell lines. Increased percentage of Cell line inhibition by suppressing viability was observed from Figure 1 -6 that a gradually increase in percentage in all the treatments. However at 150 µg/ml of tested drug doxorubicin shows 51.33±1.14 and 52.03±1.90 cell viability against IMR-32 and HT-29 cancer cell lines was observed whereas ethanol extract only crossed 50% inhibition at 200 µg/ml. The aqueous extract showed no pronounced anticancer activity compared than ethanol and acetone extracts (Figure 1-3). The ethanol extracts showed highest activity against IMR-32 cancer cell lines followed by acetone and aqueous extracts and this may be due to the greater stability of the active phytochemicals present in the solvent over a longer time (Figure 4-6). Ethanol extracts was subjected to different concentrations onIMR-32 and HT-29 cancer cell lines resulted in51.25±0.85 and 50.25±1.6% inhibition at 200 µg/ml, respectively with some significant differences ($p< 0.01$). The percentage of inhibition concentration (IC$_{50}$) is 200µg/ml. Other extracts at different concentrations shows less effect on the viability of the cancerous cell lines. Whereas aqueous extracts displayed weak inhibition against IMR-32 cancer cell lines and IC$_{50}$ is 250 µg/ml.
In this study we performed anticancer activity of aqueous, ethanol and acetone extract of A. paniculata leaves against IMR-32 and HT-29 cell lines in invitro condition. These extracts shows significant activity compared with commercial drugs. The superior activity was noted in ethanol extract compared with other extracts. Extracts of A. paniculata reduce the risk of cancer due to the presence of flavonoids (Ferguson et al 2004). Ethanol extract have alkaloids and flavonoids may have the superior activity against cancer cell lines compared with other extracts studied in this report (Vijayan et al 2004). Similarly, the results of this study are in accordance with this findings of Park et al (2008) and Reed and Pellecchial (2005) claimed that flavonoids would induce apoptosis by DNA fragmentation, nuclear condensation and cell shrinkage.

Table 1: Anticancer activity of extracts A. paniculata leaves against IMR-32 cell lines

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Standard Drug</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
<th>Acetone extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>92.53±0.99*</td>
<td>97.28±0.63**</td>
<td>93.21±1.14*</td>
<td>95.55±1.25*</td>
</tr>
<tr>
<td>100</td>
<td>76.44±1.27**</td>
<td>90.36±0.85**</td>
<td>89.69±0.65***</td>
<td>91.82±0.89***</td>
</tr>
<tr>
<td>150</td>
<td>51.33±1.14***</td>
<td>74.05±1.59*</td>
<td>60.65±1.31**</td>
<td>75.45±0.85**</td>
</tr>
<tr>
<td>200</td>
<td>29.65±0.64**</td>
<td>66.51±0.46**</td>
<td>51.25±0.85***</td>
<td>62.24±0.82***</td>
</tr>
<tr>
<td>250</td>
<td>19.23±0.81*</td>
<td>50.75±1.02*</td>
<td>32.04±0.78*</td>
<td>51.27±1.09**</td>
</tr>
<tr>
<td>300</td>
<td>10.89±0.47**</td>
<td>16.06±1.44**</td>
<td>20.98±0.95**</td>
<td>19.04±1.55*</td>
</tr>
</tbody>
</table>

* p< 0.05, ** p < 0.01, ***p < 0.001 value are considered statistically significant (BMRT)

Table 2: Anticancer activity of extracts A. paniculata leaves against HT-29 colon cancer cell lines

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Standard Drug</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
<th>Acetone extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>90.53±1.25**</td>
<td>96.28±0.63***</td>
<td>93.21±1.24***</td>
<td>94.64±1.25*</td>
</tr>
<tr>
<td>100</td>
<td>80.34±1.65*</td>
<td>89.39±0.85***</td>
<td>89.69±1.65**</td>
<td>88.75±1.97**</td>
</tr>
<tr>
<td>150</td>
<td>52.03±1.90***</td>
<td>72.25±1.59*</td>
<td>60.65±1.75*</td>
<td>70.45±1.85*</td>
</tr>
<tr>
<td>200</td>
<td>32.25±0.94***</td>
<td>56.75±0.46***</td>
<td>50.25±1.65***</td>
<td>55.24±1.82***</td>
</tr>
<tr>
<td>250</td>
<td>25.3±0.81***</td>
<td>35.65±1.02*</td>
<td>30.04±1.78**</td>
<td>41.65±1.09*</td>
</tr>
<tr>
<td>300</td>
<td>09.75±0.50**</td>
<td>26.06±1.44**</td>
<td>18.45±1.95***</td>
<td>21.58±1.55**</td>
</tr>
</tbody>
</table>

* p< 0.05, ** p < 0.01, ***p < 0.001 value are considered statistically significant (BMRT)
Figure 1: Anticancer activity of water extracts *A. paniculata* leaves against IMR-32 cell lines

Figure 2: Anticancer activity of ethanol extracts *A. paniculata* leaves against IMR-32 cell lines

Figure 3: Anticancer activity of acetone extracts *A. paniculata* leaves against IMR-32 cell lines
Figure 4: Anticancer activity of water extracts *A. paniculata* leaves against HT-29 colon cancer cell lines.

Figure 5: Anticancer activity of ethanol extracts *A. paniculata* leaves against HT-29 colon cancer cell lines.

Figure 6: Anticancer activity of water extracts *A. paniculata* leaves against HT-29 colon cancer cell lines.
CONCLUSION
In conclusion, the report of this study shows that the different extracts of *A. paniculata* leaves was toxic to cancer cell lines. Anticancer activity of water, ethanol and acetone extracts of *A. paniculata* leaves depends on the solvent used for extracting phytochemicals which present in the leaves. Ethanol extract shows more inhibition of cells when compared than other extracts may due to the presence of alkaloids and flavonoids. Minimum inhibitory concentration was observed based on the percentage of cell viability is 50% at 200 µg/ml for ethanol extracts and 250 µg/ml for water and acetone extracts against IMR-32 cell lines. Based on this results, water, and ethanol and acetone extracts of *A. paniculata* leaves potentially to be developed as herbal medicine which replace the chemotherapeutic agent against IMR-32 and HT-29 cancer cell lines.

REFERENCES


