ANTICANCER ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF
SESBANIA GRANDIFLORA AGAINST NEUROBLASTIMA (IMR-32)
AND COLON (HT-29) CELL LINES

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ABSTRACT

Herbal medicine is established on the plants contains natural chemical substances that can promote health and have curative properties for illness and diseases. Medicinal herbs play an important role in the treatment of cancer. In this study we reported that potential anticancer activity of Sesbania grandiflora leaf extracts and compared with standard commercial anticancer drug. Water, ethanol and acetone extract of S. grandiflora leaves showed invitro anticancer activity against different human cancer cell lines like neuroblastima (IMR-32), and colon (HT-29). The potential of anticancer property of S. grandiflora leaf extract was assayed by MTT method. The activity was done at different concentration like 50-300 μg/ml of the extract. From the analysis the 50% inhibitory concentration (IC₅₀) is 200 μg/ml against neuroblastima (IMR-32), and colon (HT-29) cell lines for all the extracts. While increasing the concentration of extracts showed decrease in cell viability. Extracts of S. grandiflora showed dose dependent reduction of cell viability and induction of apoptosis in the neuroblastima (IMR-32), and colon (HT-29) cell lines. This in vitro outcomes suggest a significant clinical effects of S. grandiflora against human neuroblastima (IMR-32), and colon (HT-29) cell lines.
KEYWORDS: Anticancer activity, human neuroblastima, Cell lines, *Sesbania grandiflora*

INTRODUCTION
Cancer is a worldwide public health problem and one of the leading cause of death. Cancer is defined as an irregular growth of cells exhibited uncontrolled division unconventionally resulting a gradually increase in the number of cell dividing (Kanchana and Balakrishna 2011). The development of therapies for rapidly spreading cancer is not been successful and increasing demands (Unno et al 2005; Xu et al 202009). So it is a challenge to develop a drugs for the various types of diseases. HT29 cells are human epithelial cells which produce the secretory component of Immunoglobulin A (IgA), and carcinoembryonic antigen(CEA) (Devi and Bhimba 2012). Neuroblastoma is a common type of cancer in infants that affects infants and young children formed by neuroblasts nerve cells. These immature cells grow and mature into functioning the nerve cells. But in they become cancer cells instead. Chemotherapy and radiation therapy is available for treatment and control of cancer cells but still it is exhibits low specificity and restricted by dose limiting toxicity.

Medicines from plants have played an important role in maintaining human health and improving the quality of human (Ismail et al 2012). In recent years, an increasing number of natural products have been reported to display anti-tumor compounds have been isolated from herbal plants used in various traditional medicinal systems. Herbal medicines are expected hopefully to revolutionize the cancer diagnosis and therapy. Most number of plants and their isolated constituents have been shown to potential anticancer activity (Cragg and Newman, 2005). Several plants have pharmacological properties shown to have potential to cure human cancers without causing side effects due to they have anti-tumor substances (Rani et al 2011).

*Sesbania grandiflora* is an Indian medicinal plant which is extensively used in Ayurveda and other alternative system of medicine. It is commonly known as "Sesbania" and "agathi," is widely used in Indian traditional medicine for the treatment of a wide-ranging of diseases like rheumatism, cancer and liver disorders. The plant *Sesbania grandiflora* belongs to the family Fabaceae (Kachroo et al 2011). The plant leaves serves as a natural antioxidant activity and the juice of leaves used to treat worms, biliousness, fever, gout, and leprosy (Okonogi et al., 2007). Leaves of this plant has medicinal effect due to its astringency property; hence it is used against inflammation, venom and other poisons, bacterial infections and tumors (Mannetje and Jones 1992). Recently, *Sesbania grandiflora* (antianxiety)
anxiolytic, hepatoprotective, cardio, antiurolithiatic and antioxidant activities were reported. Extract of *S. grandiflora* leaves shows significant hepatoprotective (Kasture 2002), antimicrobial (Pari and Uma 2003), analgesic and antipyretic activity (Vijay et al 2009). Due to the large use of *S. grandiflora*, the objective of this study was to investigate the anticancer activity of its leaf water, ethanol and acetone extracts against neuroblastoma (IMR-32), and colon (HT-29) cell lines at dose dependent method.

**MATERIAL 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.

**Preparation of extraction of *Sesbania grandiflora* leaves**

Plant leaves were collected from Arcot, Vellore Dist, TN, India. The collected plant leaves were shade dried and powdered. The powdered materials will be packed and extracted with 80% ethanol and acetone in two Soxhlet apparatus for 24 hrs at 55°C. Water extract was prepared by immersing 100 g dried leaf powder into 200 ml double distilled for 24 hours. The extracts will be concentrated using rotary flash evaporator and the extract will be used to evaluate anticancer activity.

**Anticancer activity against neuroblastoma (IMR-32) and human colon (HT-29) cancer cell line**

Cells viability test was done by the MTT(3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazoliumbromide) assay is s colorimetric analysis based on the measuring the activity of cellular enzymes that living cells were reduce the yellow MTT dye into insoluble purple color formazan. Cells were plated and grown with different concentration of plant extracts and incubated for 24 hours in CO2 atmosphere. After 24 hours treatment, MTT was added in each well and incubated at 37°C for 4 hours in 5% CO2 chamber. Then the medium was removed and washed with Phosphate buffer solution. Then, DMSO was added to each well which dissolve the insoluble formazan crystals into colored solution. The intensity of the colored solution was measured using ELISA microplate reader at 570 nm. The results were expressed as the percent optical density of treated cells to that of the control cells. 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 control. The assay was performed in triplicate for each extracts.

\[
\text{Absorbance for treated cells} \\
\text{% Cell viability} = \frac{1}{\text{Absorbance for control cells}} \times 100
\]

**Statistical analysis**

The quantitatively obtained data were analyzed using one way Analysis of Variance (ANOVA) and expressed as mean± S.E.M. value of p< 0.05 is considered as Statistical significant. The experiment data plots of the cell viability against drug and extract concentration.

**RESULTS AND DISCUSSION**

The medicinal herb S. garandiflora leaves showed anticancer activity against neuroblastima (IMR-32), and colon (HT-29) cell lines (Table 1 and 2). In the present study, the treatment with water, ethanol and acetone extracts suppressed the cell viability up to 50% at 200µg/ml against both cell lines (Figure 1-6). Plant extracts show more significant activity as compared to the positive control. The extract showed significant inhibition in the cell viability in a dose dependent manner. The treatment with ethanol extract against neuroblastima (IMR-32), and colon (HT-29) cell lines significantly decrease the viability of cells at 200g/ml when compared other extracts. The cells were contact with 50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml, 250µg/ml, and 300µg/ml of extract showed decreased number of cell viability. The results indicate that extracts of S. grandiflora has an anticancer activity in both cell lines. The maximum cytotoxic effect was observe in ethanol extract. This variation in activity occurs due to presence of different phyto-constituents like flavonoids, alkaloids and steroids. Alkaloids (Yang and Wang, 1993) Flavonoids, (conese and Blasi, 1995), phenols, polyphenols and other derivatives have been associated with anticancer property (Cirla and Mann, 2003). Ethanol extract has high amount of alkaloids and flavonoids which actively involved in the cancer cell death. Cell death occur by apoptosis and necrosis caused by the drug. The phychemicals alkaloids, flavonoids and polyphenols were actively inhibit the cells in the protein synthesis either by damaging DNA or by blocking at transnational level which may be determine the mortality of cells (Singh et al 2013).
Table 1: Anticancer activity of extracts *S. grandiflora* 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>98.12±0.99*</td>
<td>94.21±1.14**</td>
<td>96.55±1.25**</td>
</tr>
<tr>
<td>100</td>
<td>76.44±1.27**</td>
<td>92.56±1.09*</td>
<td>84.87±1.65**</td>
<td>91.82±1.64*</td>
</tr>
<tr>
<td>150</td>
<td>51.33±1.14*</td>
<td>79.78±1.25**</td>
<td>66.65±1.25**</td>
<td>65.65±1.45**</td>
</tr>
<tr>
<td>200</td>
<td>29.65±0.64*</td>
<td>57.19±1.16**</td>
<td>52.15±0.85***</td>
<td>54.25±0.82**</td>
</tr>
<tr>
<td>250</td>
<td>19.23±0.81**</td>
<td>40.54±1.15**</td>
<td>42.15±1.09**</td>
<td>45.77±1.9**</td>
</tr>
<tr>
<td>300</td>
<td>10.89±0.47*</td>
<td>26.38±0.95**</td>
<td>21.64±0.95***</td>
<td>23.61±0.95**</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 *S. grandiflora* 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>94.65±1.76**</td>
<td>91.2±0.94**</td>
<td>93.15±1.65**</td>
</tr>
<tr>
<td>100</td>
<td>80.34±1.65*</td>
<td>86.33±1.24**</td>
<td>82.56±1.15**</td>
<td>84.65±1.54**</td>
</tr>
<tr>
<td>150</td>
<td>52.03±1.90**</td>
<td>74.15±1.52*</td>
<td>62.85±1.87***</td>
<td>75.45±1.25***</td>
</tr>
<tr>
<td>200</td>
<td>32.25±0.94**</td>
<td>58.75±1.06**</td>
<td>51.35±1.75***</td>
<td>57.43±1.02**</td>
</tr>
<tr>
<td>250</td>
<td>25.30±0.81**</td>
<td>45.65±1.92**</td>
<td>32.84±1.35**</td>
<td>39.27±1.17**</td>
</tr>
<tr>
<td>300</td>
<td>09.75±0.50*</td>
<td>36.96±1.34*</td>
<td>20.45±1.95**</td>
<td>21.25±1.45*</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 *S. grandiflora* leaves against IMR-32 cell lines
Figure 2: Anticancer activity of ethanol extracts *S. grandiflora* leaves against IMR-32 cell lines

Figure 3: Anticancer activity of acetone extracts *S. grandiflora* leaves against IMR-32 cell lines

Figure 4: Anticancer activity of water extracts *S. grandiflora* leaves against HT-29 colon cancer cell lines
CONCLUSION
The synthetic or semisynthetic medicines can cure the diseases but at the same time they are highly toxic in nature, whereas the herbal drug are minimize the adverse side effects. In this present report we concluded that anticancer activity of different solvent derived extracts of *S. grandiflora* like water, ethanol, and acetone determined by colorimetric method of MTT assay. Live cells reduce the MTT yellow dye into insoluble purple color formazan at 50% in
200µg/ml concentrations. The IC$_{50}$ concentration is 200 µg/ml for all the extracts. Hence, our report suggested that the herbal medicine from *S. grandiflora* leaves actively in the growth of cancer cells better than standard drug would be replace chemotherapy treatment. Further study needed to identify the exact active compound present in the *S. garandiflora* leaves underlying this high anticancer activity.

**REFERENCES**


