In-vitro Anticancer Activity of Ethanolic Extract of Leaf of Pleiospermium alatum in MCF-7, MDA-MB-231, Hela, HepG2 Cell Lines

R Elumalai1, V Ravichandiran1

Abstract: The anticancer-cytotoxic activities of ethanolic extract of Pleiospermium alatum were tested under in-vitro conditions in different cancer cell lines like MCF-7, MDAMB-231, Hela, HepG2 cell lines. The active constituents carbohydrates, steroids, Alkaloids, Tannins, Flavonoids, Saponins, gums and mucilage. The ethanolic extract of Pleiospermium alatum sowed a good cytotoxic activity (60%,60%,85% and 65%) in Hela, HepG2, MCF-7, MDAMB-231 cells at 24 hrs respectively. In this in-vitro studies the normal cells are act as a positive control. The ethanolic extract of Pleiospermium alatum shows maximum percentage of 85% after 24 hrs. From this study, it can be concluded that the phytoconstituents of Pleiospermium alatum have significant anti cancer-cytotoxic activity on Hela, HepG2, MCF-7, MDAMB-231 cells.

INTRODUCTION
Cancer is one of the most prominent diseases in humans. Currently, there is considerable scientific and commercial interests in continuing for the discovery of new anticancer agents from natural products. [1] The potential of natural products as anticancer agents has been first recognized in years 1950s by the U.S. National Cancer Institute (NCI), and since then has made the major contributions to the discovery of new naturally occurring anticancer agents. [2]

The development of the vinca alkaloids, vinblastine and vincristine and the isolation of the cytotoxic podophyllotoxins as anticancer agents are good examples for new agents from plant sources. This has led to the followed researches of many novel chemotypes showing a range of cytotoxic activities. [3] Herbal medicine is defined as branch of science in which plant based formulations are used. To alleviate the diseases. It is also known as botanical medicine or phytomedicine. In the early twentieth century herbal medicine was prime healthcare system as antibiotics and analgesics were not available. [4] Herbal medicines are playing a vital role in the treatment of various chronic diseases when compared to modern synthetic drugs. They are very safe because of its minimum side effects. Proper knowledge of crude drug is very important aspect in the development of herbal formulation, safety and efficacy of the herbal product. Pharmacognosy, a science which deals with an entire information of the crude drug obtained (Wall ex Wight and Arn) Swingle belongs to the family Rutaceae. It is widely grown in India. Pleiospermium alatum, Swingle (family: Rutaceae), is commonly called as “Kurunthumul thazhai”, is a medicinal plant distributed in India (Punjab, Bihar, Orissa, Assam, Madhya Pradesh, Bombay, Mysore, and Tamil Nadu), Burma, Thailand, South Western China, Indochina and Ceylon. Flowers are white and fragrant; orange like fruits are having bitter taste. [5] The leaves and bark are used for the fomentation of rheumatic pain; the dried fruit is useful in malignant and pestilent fevers and is used as an antidote for poisons. [6] The various parts of this plant contains polyphenolic compound Apigenin - 7 - O - diglucoside. [7] acridone alkaloids acrid-9-one, acronycine and coumarin derivatives aurapten, suberenol, umbelliferone, xanthoxyletin, skimmnin, apiosyl and seselin-10,19-ABE0-7-α-acetoxy-10-β-hydroxy aurapten. [8, 9] From the literature details, different activities have been attributed to this plat. To our knowledge no report on the effect of this plant in experimental cytotoxicity studies. This study was therefore undertaken to evaluate the effect of ethanolic extract of the leaf of Pleiospermium alatum on different cancer cell lines like MCF-7, MDA-MB-231, Hela, HepG2 cell lines.

MATERIALS AND METHODS
Collection of Plant Materials
The plant materials (leaves) of Pleiospermium alatum (Wight & Arn.) Swingle were collected from the Jammamardhur forest vicinity, of Tiruvannamalai district. The collected plant materials were botanically identified and confirmed by the Botanist Dr. A. C. Tangavelou, Director, Bio-Science Research Foundation, Pondicherry. The herbarium specimen was prepared and deposited at Bio-Science Research Foundation, Pondicherry, for future reference.

Preparation of the Extracts
The collected materials (leaves) were chopped into small pieces separately, shade-dried, and coarsely powdered (sieve no. 40) using a pulverizer. The coarse powders were subjected to successive extraction with organic solvents such as petroleum ether, chloroform and ethanol by Soxhlet method. [10] The extract was collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was removed in vacuum. The resulted extracts were used for phytochemical screening.

Preliminary Phytochemical Screening
All the extracts were subjected to preliminary phytochemical tests as per standard procedure. [10-11]

1. Test for Alkaloids
To the test solution, add Mayor’s reagents (Potassium mercuric iodide)/wagner’s reagent (solution of Iodine in Potassium iodide)/Dragendorff’s reagent (Potassium mercuric nitrate) and compare with the known standards. 

1Department of Pharmacognosy, School of Pharmaceutical Sciences, Vels University, P V Vathiyalingam Road, Velan Nagar, Pallavaram, Chennai-600117, Tamil Nadu, India.
E-mail: sasimalai1965@gmail.com
*Corresponding author
iodide, bismuth nitrite)/Hager’s reagent (Picric acid), a
creamish precipitate or brown precipitate or brownish red
precipitate or orange/yellow precipitate indicates the
presence of alkaloids.

2. Test for Anthraquinones
To the test solution, add a drop of benzene and a drop of
ammonia, a pink color develops which indicate the
presence of Anthraquinones.

3. Test for Flavonoids
Three methods were used to determine the presence of
flavonoids in the plant sample.

5 ml of dilute ammonia solution was added to a portion
of the aqueous extract of the plant followed by addition of
concentrated sulphuric acid. A yellow coloration observed
indicates the presence of flavonoids. The yellow coloration
disappeared on adding few drops of 1% ammonia solution.
The portion of the powdered plant sample was heated
with 10 ml of ethyl acetate over a steam bath for 3 min. The
mixture was filtered and 4 ml of the filtrate was shaken
with 1 ml of dilute ammonium solution. A yellow color was
observed indicates the presence of flavonoids.

To the test solution in 10 ml ethanol add concentrated
hydrochloric acid and magnesium ribbon it develops a
pink-tomato red (magenta) colour indicates the presence of
flavonoids.

4. Test for Coumarins
To the test solution, add a drop of sodium sulphate which
turns into yellow color, indicates the presence of
coumarins.

5. Test for Phenols
To the test solution add a drop of ferric chloride; violet/purple
color develops which indicates the presence of phenols.

6. Test for Saponins
About 2 g of the powdered sample was boiled in 20 ml of
distilled water in a water bath and filtered. 10ml of the filtrate
was mixed with 5 ml of distilled water and shaken vigorously
for a stable persistent froth which indicates the presence of
saponins.

7. Test for Steroids (Liebermann-burchard Test)
Two ml of acetic anhydride was added to 0.5 g ethanolic
extract of each sample with 2 ml sulphuric acid. The colour
changed from violet to blue/green indicates the presence of
steroids.

8. Test for Tannins
About 0.5 g of the dried powdered samples was boiled in
20 ml of water in a test tube and then filtered. A few drops
of 0.1% ferric chloride was added and observed for
brownish green/blue-black colouration.

9. Test for Terpenoids (Salkowski Test)
Five ml of each extract was mixed in 2 ml of chloroform,
and concentrated sulphuric acid (3 ml) was carefully added
to form a layer. A reddish brown colouration of the inter-
face was formed to show positive results for the presence of
terpenoids.

10. Test for Carbohydrates:
   a. Reducing Sugar (Fehling’s Test)
   Mix 1 ml each of Fehling's solutions A and B in a test tube
   and add the mixture to test tube B. Heat the content of
   the test tube on a water bath. The formation of a reddish
   brown precipitate indicates the presence of reducing sugar.

   b. Non-reducing Sugar (Benedict’s Test)
   Add 1 ml of Benedict’s reagent to test tube C and heat the
   mixture to boiling in a water bath for 2 minutes. The
   formation of a orange red precipitate due to the formation
   of copper (I) oxide indicates the presence of reducing sugar.

11. Test for Catechins
To the sample solution, add Ehrlich's reagent. Reddish
purple/pink colour develops. This indicates the presence of
catechin.

12. Test for Amino Acids and Proteins (Ninhydrin
    Reaction)
Take 2-3 ml of sample solution in a test tube. Add 3-4 drops
of ninhydrin solution to it and heat. Appearance of purple
or violet color indicates the presence of protein.

Physico-Chemical Analysis
The powdered plant materials were morphologically and
organoleptically screened and subjected to physico-
chemical analysis in accordance with the WHO guidelines
(WHO, 1998). The various parameters considered were

1. Determination of Ash Values
Ash values of a crude drug is the inorganic residue
remaining after incineration, which simply represents
inorganic salts, naturally occurring in drug or adhering to it
or deliberately added to it as a form of adulteration. Hence,
the ash values are useful in determining the quality and
purity of a crude drug in the powdered form.

2. Determination of Total Ash
Weighed 2 to 3 g of the air-dried crude drug in a tared
platinum or silica dish and incinerated at a temperature not
exceeding 450°C until free from carbon, cooled and
weighed. Calculated the % of total ash with reference to the
air-dried drug.

3. Determination of Water Soluble Ash
Boiled the ash, (obtained in ash value determination), for 5
minutes with 25 ml of water; collected the insoluble matter
in a Gooch crucible or on an ashless filter paper, washed
with hot water, and ignited for 15 minutes at a temperature
not exceeding 450°C. Subtracted the weight of the insoluble
matter from the weight of the ash; the difference in weight
represented the water-soluble ash. Calculated the % of
water-soluble ash with reference to the air-dried drug.
4. Determination of Acid Insoluble Ash
Boiled the ash, (obtained in ash value determination), with 25 ml of 2 M hydrochloric acid for 5 minutes, collected the insoluble matter in a Gooch crucible or on an ashless filter paper, washed with hot water, ignited, cooled in a desiccator and weighed. Calculated the % of acid-insoluble ash with reference to the air-dried drug.

5. Determination of Sulphated Ash
Heated a silica or platinum crucible to redness for 10 minutes, allowed to cool in a desiccator and weighed. Unless otherwise specified in the individual monograph, transferred to the crucible 1 g of the substance being examined and weighed the crucible and the contents accurately. Ignited, gently at first, until the substance is thoroughly charred. Cooled, moistened the residue with 1 ml of sulphuric acid, heated gently until the white fumes are no longer evolved and ignited at 800±25°C until all black particles have disappeared. Conducted the ignition in a place protected from air currents. Allowed the crucible to cool, added a few drops of sulphuric acid and heated. Ignited as before, allowed to cool and weighed. Repeated the operation until two successive weighings did not differ by more than 0.5 mg.

6. Determination of Loss on Drying
The loss on drying test is designed to measure the amount of water and volatile matters in a sample, when the sample is dried under specific conditions. If the substance is in the form of large crystals, reduce the size by rapid crushing to a powder. The test should be carried out on a well mixed sample of the substances.

Weighed a glass stoppered, shallow weighing bottle that has been dried under the same conditions to be employed in the determination. Transferred to the bottle the quantity of the sample specified in the related monograph, covered it and accurately weighed the bottle and the contents. Distributed the sample as evenly as practicable by gentle sidewise shaking to a depth not exceeding 10 mm.

Placed the loaded bottle in the drying chamber (oven or desiccator) as directed in the monograph, removed the stopper and left it also in the chamber. Dried the sample to constant weight or for the specified time and at the temperature indicated in the monograph. After drying was completed, opened the drying chamber, closed the bottle promptly and allow it to cool at room temperature (where applicable) in a desiccator before weighing. Weighed the bottle and the contents.

Fluorescence Analysis
1. In Chemical Reagents
The analysis of powder and extract under day light is unreliable due to lack of fluorescence. So it is evaluated under near and far UV.

2. Methodology
The Fluorescence analysis of leaf powder was carried out by mixing the powder with various chemical reagents and observing it under daylight, near UV and far UV.
RESEARCH ARTICLE

attributed to the competent cellular uptake and intracellular distribution of extract compounds.

Effect of Ethanolic Extract of Pleiospermium alatum in In-vitro Cytotoxicity of HepG2 Cells Treated with Different Concentration

The in vitro cytotoxicity of treated and non-treated cells were investigated using MTT cell viability assay against HepG2 cell line. The cytotoxicity of a treated ethanolic extracts the increasing concentrations were analysed. The killing effect of the ethanolic extract was more pronounced at the low concentrations (5μg/ml) and exhibited higher cytotoxicity when compared. When the experimental cells were exposed to the highest concentration 40 μg/ml for the period of 24 h, 60% of cells were killed by ethanolic extract (Figure 2). The higher cytotoxic effect of ethanolic extracts could be attributed to the competent cellular uptake and intracellular distribution of extract compounds.

Effect of Ethanolic Extract of Pleiospermium alatum in In-vitro Cytotoxicity of MCF-7 Cells Treated with Different Concentration

Table 1: Preliminary Phytochemical Screening of Hydro-alcoholic Extract of Pleiospermium alatum

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Hydro-alcoholic Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>–</td>
</tr>
<tr>
<td>Aminoacid and Proteins</td>
<td>–</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>–</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Catechins</td>
<td>–</td>
</tr>
<tr>
<td>Coumarins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Gum, Oil &amp; Resins</td>
<td>+</td>
</tr>
<tr>
<td>Phenolics</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>–</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 2: Physico-chemical Parameters of the Leaves of Pleiospermium alatum

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total ash</td>
<td>5.7%</td>
</tr>
<tr>
<td>2</td>
<td>Water soluble ash</td>
<td>4.3%</td>
</tr>
<tr>
<td>3</td>
<td>Sulphated ash</td>
<td>5.2%</td>
</tr>
<tr>
<td>4</td>
<td>Acid insoluble ash</td>
<td>3.7%</td>
</tr>
<tr>
<td>5</td>
<td>Loss on Drying</td>
<td>2.9%</td>
</tr>
</tbody>
</table>

Table 3: Fluorescence Analysis of Leaves of Pleiospermium alatum in Various Solvents

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvents</th>
<th>Day light</th>
<th>UV light</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether</td>
<td>Green</td>
<td>Pale Fluorescent green</td>
</tr>
<tr>
<td>2</td>
<td>Hexane</td>
<td>Green</td>
<td>Pale Fluorescent green</td>
</tr>
<tr>
<td>3</td>
<td>Benzene</td>
<td>Pale green</td>
<td>Fluorescent green</td>
</tr>
<tr>
<td>4</td>
<td>Chloroform</td>
<td>Green</td>
<td>Dark green</td>
</tr>
<tr>
<td>5</td>
<td>Ethyl acetate</td>
<td>Green</td>
<td>Fluorescent green</td>
</tr>
<tr>
<td>6</td>
<td>Acetone</td>
<td>Dark green</td>
<td>Fluorescent green</td>
</tr>
<tr>
<td>7</td>
<td>Ethanol</td>
<td>Dark Green</td>
<td>Fluorescent green</td>
</tr>
<tr>
<td>8</td>
<td>Methanol</td>
<td>Dark Green</td>
<td>Fluorescent green</td>
</tr>
<tr>
<td>9</td>
<td>Water</td>
<td>Pale Brown</td>
<td>Dark Green</td>
</tr>
</tbody>
</table>

Table 4: Fluorescence Analysis of Leaves of Pleiospermium alatum in Various Chemical Reagents

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Chemical Reagents (50%)</th>
<th>Day light</th>
<th>UV light</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hydrogen peroxide</td>
<td>Reddish brown</td>
<td>Pale green</td>
</tr>
<tr>
<td>2</td>
<td>Nitric acid</td>
<td>Golden yellow</td>
<td>Fluorescent green</td>
</tr>
<tr>
<td>3</td>
<td>Hydrochloric acid</td>
<td>Pale green</td>
<td>Fluorescent green</td>
</tr>
<tr>
<td>4</td>
<td>Ferric chloride</td>
<td>Reddish brown</td>
<td>Dark green</td>
</tr>
<tr>
<td>5</td>
<td>Ammonia</td>
<td>Dark brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>6</td>
<td>Iodine</td>
<td>Brownish Red</td>
<td>Dark brown</td>
</tr>
<tr>
<td>7</td>
<td>Fehling’s solution</td>
<td>Orange</td>
<td>Green</td>
</tr>
<tr>
<td>8</td>
<td>Thionyl chloride</td>
<td>Orange</td>
<td>Green</td>
</tr>
</tbody>
</table>
The in vitro cytotoxicity of treated and non-treated cells were investigated using MTT cell viability assay against MCF-7 cell line. The cytotoxicity of a treated ethanolic extract with increasing concentrations was analysed. The killing effect of the ethanolic extract was less pronounced at the low concentrations (5 μg/ml) and exhibited higher cytotoxicity at (15 μg/ml) when compared to methanolic extract. However, the difference was still significant even at the higher concentrations (45 μg/ml). When the experimental cells were exposed to the highest concentration 50 μg/ml for the period of 24 h, 85% of cells were killed by ethanolic extract (Figure 3). The higher cytotoxic effect of ethanolic extracts could be attributed to the competent cellular uptake and intracellular distribution.

**Effect of Ethanolic Extract of Pleiospermium alatum in In vitro Cytotoxicity of MCF-7 Cells Treated with Different Concentration**

The in vitro cytotoxicity of treated and non-treated cells were investigated using MTT cell viability assay against MDA-MB-231 cell line. The cytotoxicity of a treated ethanolic extract with increasing concentrations were analysed. The killing effect of the ethanolic extract was more pronounced at the low concentrations (1 μg/ml) and exhibited higher cytotoxicity at (10 μg/ml) when compared to ethanolic extract. However, the difference was still significant even at the higher concentrations (10 μg/ml). When the experimental cells were exposed to the highest concentration 10 μg/ml for the period of 24 h, 65% of cells were killed by ethanolic extract (Figure 4). The higher cytotoxic effect of ethanolic extracts could be attributed to the competent cellular uptake and intracellular distribution.

**DISCUSSION**

The plant *pleiospermium alatum*, belonging to family Rutaceae have been used for very long times in traditional system of medicine as anti-inflammatory agent and in wound healing. It is widely grown in India. Besides the
traditional uses the plant were reported numerous pharmacological effects viz. anti-inflammatory, antimicrobial activity. Beside this studies were carried out by various researchers to showing importance of *Pleiospermium alatum*. As per phytochemical study the alkaloidal, phenolic, flavonoidal content with various phytoconstituents were present. Phytoconstituents possessing anti-oxidant properties are believed to prevent or slow down the occurrence of disease such as cancer. Qualitative Phytochemical screening concluded that ethanolic extract of *Pleiospermium alatum* contains alkaloids, steroid and carbohydrates, flavonoids, tannins. The objective of the present study was to investigate the *in vitro* anticancer properties of *Pleiospermium alatum* (leaves). *In-vitro* anticancer activity was performed on the ethanolic extract of *Pleiospermium alatum*. The MTT reagent assay have wide limitations over SRB assay because formation of colour in MTT relies on the activity of reagent assay have wide limitations over SRB assay. Bottom of the table is similar result may be given as if the cells were not alive or not proliferating. *In-vitro* anticancer activity was performed on 4 different human cell lines i.e., lung, liver, breast cancer using MCF-7, MDAMB-231, Hela, HepG2 cell lines by using MTT assay. The ethanolic extracts of *Pleiospermium alatum* leaves were shown the significant cytotoxicity in different cancer cell lines. The results were discussed in detail. Now overall study evaluate that *Pleiospermium alatum* has potential activity on MCF-7, MDAMB-231, Hela, HepG2 cell lines compared with normal cells. So these drug has considerable anticancer activity.

**CONCLUSION**

*Pleiospermium alatum* (Rutaceae) family shows the significant anticancer activity in invitro studies using MTT assay using different cancer cell lines like MCF-7, MDAMB-231, Hela, HepG2 cell lines. So, further studies are warranted to do the invivo studies and isolate and characterize the phytoconstituents.

**REFERENCES AND NOTES**
