EVALUATION OF ANTIMICROBIAL AND ANTICANCER ACTIVITY OF CATHARANTHUS ROSEUS.

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ABSTRACT

Catharanthus roseus is one of the medicinal native Madagascar and commonly distributed in various regions of Asian continent and worldwide. The plant is used as an anti-helminthic, anti-ulcer, hypotensive, anti-diarrheal, phytoremediation, antitumor etc., The present study aims to evaluate the anticancer activity of ethanolic extract of Catharanthus roseus leaves on the human hepatocellular carcinoma cell line, antimicrobial properties and the phytochemical analysis of the extract. A phytochemical screening of the major constituents of the aforesaid plant revealed the presence of tannins, flavonoid, alkaloid, quinone, terpenoid, coumarins. Further, the ethanolic extract was tested for its anti-microbial activities where the maximum zone of inhibition (21mm) was observed in Escherichia coli at a concentration of 5mg/ml and 16mm zone of inhibition against Bacillus subtilis at a concentration of 5mg/ml. MTT assay confirmed the anticancer activity of the ethanolic extract. Thus antimicrobial and anticancer potency of the leaves of Catharanthus roseus was proved.

KEYWORDS: Catharanthus roseus, phytochemical screening, antibacterial, anticancer, MTT assay.

INTRODUCTION

Cancer is a major public health burden in both developed and developing countries, occurring due to some molecular changes within the cell. World Health Organization (WHO) has classified cancer among non-communicable diseases, which are responsible for 63% of...
deaths worldwide. (Alwan et al., 2010). The World Bank income groups estimated that the incidence of 12.7 million new cancer cases in 2008 (Parkin et al., 2005) will rise to 21.4 million by 2030, and low or middle-income countries will be the most affected with nearly two thirds of all cancer diagnoses on Cancer. It was estimated that there were 10.9 million new cases, 6.7 million deaths, and 24.6 million persons living with cancer around the world in 2002. (Parkin et al., 2005). It becomes the second major cause of death in the human after cardiovascular disease (Jackson, 2000). The major causes of cancer are smoking, dietary imbalances, hormones and chronic infections leading to chronic inflammation.

According to WHO estimates, 80% of the rural population of this region has almost exclusively uses traditional medicine for its needs of primary health care (Farnsworth et al., 1985). This massive use of traditional medicine, composed mainly of medicinal plants, is related to cultural and economic reasons. This is why WHO encourages countries of this region to promote and integrate traditional medical practices in their health system. (Ashidi et al., 2010 and Awodele et al., 2011) A large number of these medicinal plants are also used in several formulations for the treatment of various diseases caused by microbes. A wide range of medicinal plant parts are used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries (Uniyal et al., 2006). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not been adequately evaluated (Balandrin et al., 1985).

*Catharanthus roseus* called as ‘Periwinkle’ in English is a common ornamental plant grown in the gardens of residential and official compounds. This plant have proved to be significant natural resources for effective chemotherapeutic agents and offering a broad spectrum of activity with greater emphasis on preventive action (ANOOP, 2015). Thus, this study aims to investigate some of the anti-microbial properties of this plant. The anticancer properties of Catharanthus roseus has been the major interest in all investigations.
MATERIALS AND METHODS

Plant collection
The *Catharanthus* plant was collected from nearby residential area of Velachery, Chennai, Tamil Nadu, India. The leaves were separated from other parts, washed, cleaned and dried for further use.

Extraction and Isolation
The dried leaf materials were washed, air-dried at room temperature (26°C) for 2 weeks, after which it was ground to a uniform powder. The dry powder was extracted by reflexed in 100 mL methanol for 24 h, using a Soxhlet apparatus (Khan et al., 1988). The extract was filtered using Whatman filter paper, No. 1. The filtrate was then evaporated using rotatory evaporator (Superfit-ROTAVAP, India) and dried at 55°C. Ethanol and distilled water extracts are obtained and all the extracts are preserved. Dried extract was stored at 20°C in labeled, sterile capped bottles.

Preliminary phytochemical screening
The phytochemical screening of the plant extracts was carried out according to procedure of Farnes worth (Lalitha et al., 2015).

Test Micro Organisms
Clinical isolates gram positive bacteria and gram negative bacteria were grown in nutrient broth medium and incubated at 37°C for 24 hours, followed by frequent sub culturing (every 24 hrs) to refresh medium. Bacterial strains were maintained on Muller Hinton Agar Medium. The bacteria used for the analysis were *E.coli*, *Klebsiella sp.*, *Bacillus sp.*, *Enterobacter sp.*, *Staphylococcus aureus*, *Pseudomonas sp.*

Preparation of Microbial cultures
The bacterial strains were inoculated into nutrient broth for 24 hours. In the Agar well diffusion method, sterile Muller Hinton agar for bacteria was inoculated with the test and then incubated at 37°C for 24 hrs. At the end of the period, zones of inhibition were measured in millimetres.

Antibacterial activity – Well diffusion method
Antibacterial activity of all extracts was determined on Muller & Hinton Agar (Hi-Media Pvt. Ltd. Mumbai) using Kirby-Bauer disk diffusion method (Bauer, et al., 1966). Test pathogens
were spread on the test plates- Muller Hinton agar (MHA) for bacteria using sterile swabs. Sterile wells were made with the help of a sterile cork borer at aseptic conditions. Samples (1000, 500, 250, 125 and 62.5µg) were added to the wells at aseptic conditions. Stock solutions of the extracts were prepared using DMSO. The test plates were incubated for 24hrs. The zone of inhibition (in mm diameter) were read and taken as the activity of the extract against the test organisms.

MTT assay

Cell maintenance

Human hepatocellular carcinoma (Hep3B) cell line was cultured in Dulbecco’s Modified Eagle Medium (DMEM). All culture media were supplemented with 10% fetal bovine serum (FBS), 1% antibiotic and antymycotic solution (50,000 units/L of penicillin and 50 mg/L of streptomycin) and 2 mM glutamine. Cultures were held in 75 cm2 culture flasks at 37°C, 5% CO and 95% relative humidity, changing media at least twice a week.

Procedure

A parallel set of plates was set up for the MTT assay and seeded and exposed in an identical manner. After 24 and 48 hours of Ag nanoparticle (AgNP) exposure, the medium for the control or test exposures was removed, the cells were washed with PBS and 100 µl of freshly prepared MTT in media (5 mg/ml of MTT in media [without FBS or supplements]) were added to each well. After 3hr incubation, the medium was discarded and the cells were rinsed with PBS and 100 µl of MTT fixative solution (isopropanol with 0.04 N HCl) were added to each well and the plates were shaken at 240 rpm for 10 min. The absorbance was then measured at 620 nm in an ELISA reader (Gordon et al., 2008).

RESULTS AND DISCUSSION

Yield

0.5 g of extract was obtained as yield from running the Soxhlet and evaporating the sample (Figure no. 1).

Phytochemical Tests

The qualitative phytochemical study revealed the presence of several chemical groups: tannins, flavonoids, alkaloid, quinone, glycosides, terpenoids and coumarins. This qualitative phytochemical study shows that all the chemical groups identified at level of the leaves of Catharanthus roseus find themselves in the traditional preparation (decoction).
**Antibiotic Sensitivity disc pattern using standards**

After 16 to 18 hours of incubation, each plate is examined. The resulting zones of inhibition was uniformly circular and there was a confluent lawn of growth. There was no individual colonies apparent indicating that the inoculum was not too light and dilution was right. The diameters of the zones of complete inhibition (as judged by the unaided eye, using scale) were measured by holding it back of the inverted plate, including the diameter of the disc.

The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored. The sizes of the zones of inhibition are interpreted by referring through 2I (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints) of the NCCLS M100-S12: Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement, and the organisms reported as either susceptible, intermediate, or resistant to the agents that have been tested shown in Table 1. All the organisms were resistant to ampicillin and sensitive to azithromycin and ciprofloxacin.

**Antibacterial activity –using extracts**

The antibacterial activity was carried out for the various concentrations of the extract. It was found that the maximum zone of inhibition (21mm) was observed in *Escherichia coli* at a concentration of 5mg/ml and 16mm zone of inhibition was observed against Bacillus sp at a concentration of 5mg/ml as shown in Table 2.

**MTT assay**

Invitro cytotoxicity of extract was evaluated on HepG2 cell line for proliferation and survival of HepG2 cells by exposing to 5-0.31mg/ml of test samples for 24 h. Figure 2 showed that herbal extract induced cell death in a dose- dependent manner, as determined using MTT assay. The 50% inhibition were detected at 40-60 microgram/ml. No significant change in cell viability was observed in DMSO treated group when compared with control. Thus, the results shows that the silver nanoparticles inhibits the proliferation of the cell.

**CONCLUSION**

The herbal technology industry has matured and expanded at a rapid pace in the last decade, leading to the research and development of many bioactive materials with enormous potential. Thus, in the present study *Catharanthus roseus* showed significant antimicrobial
property and effective cell death on HepG2 cell lines at various concentration. Further studies are needed in order to analyze the mechanism behind the effects observed and the future effects of \textit{Catharanthus roseus}. Our data stands as a beginning to a good future research which may involve, advanced in vivo studies on toxicity proving its more safety and toxicity. Thus supporting this primary analysis and may result in becoming a drug of choice.

![Figure 1 Catharanthus roseus leaves and extract](image)

**Table: 1 Antibiotic Sensitivity disc pattern using standards**

<table>
<thead>
<tr>
<th>ANTIBIOTIC</th>
<th>AU</th>
<th>A</th>
<th>AZ</th>
<th>TE</th>
<th>CP</th>
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<tbody>
<tr>
<td>\textit{Enterobacter}</td>
<td>-</td>
<td>-</td>
<td>30 mm</td>
<td>20 mm</td>
<td>25 mm</td>
</tr>
<tr>
<td>\textit{E.coli}</td>
<td>10 mm</td>
<td>-</td>
<td>25 mm</td>
<td>11 mm</td>
<td>13 mm</td>
</tr>
<tr>
<td>\textit{Bacillus}</td>
<td>-</td>
<td>-</td>
<td>14 mm</td>
<td>-</td>
<td>20 mm</td>
</tr>
<tr>
<td>\textit{Staphylococcus aureus}</td>
<td>10 mm</td>
<td>-</td>
<td>24 mm</td>
<td>26 mm</td>
<td>35 mm</td>
</tr>
<tr>
<td>\textit{Pseudomonas}</td>
<td>22 mm</td>
<td>20 mm</td>
<td>32 mm</td>
<td>19 mm</td>
<td>30 mm</td>
</tr>
<tr>
<td>\textit{Klebsiella}</td>
<td>9 mm</td>
<td>-</td>
<td>24 mm</td>
<td>18 mm</td>
<td>34 mm</td>
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</tbody>
</table>

AU- Amoxicillin ; A – Ampicillin; Az –Azithromycin; TE – Tetracycline, CP – Ciprofloxacin

**Table: 2 Antibacterial activity – well diffusion method**

<table>
<thead>
<tr>
<th>ANTIMICROBIAL ACTIVITY</th>
<th>5mg/ml</th>
<th>2.5mg/ml</th>
<th>1.25mg/ml</th>
<th>0.62mg/ml</th>
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<td>8 mm</td>
<td>3 mm</td>
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<tr>
<td>\textit{E.coli}</td>
<td>21 mm</td>
<td>17 mm</td>
<td>6 mm</td>
<td>-</td>
<td>-</td>
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<tr>
<td>\textit{Bacillus}</td>
<td>16 mm</td>
<td>12 mm</td>
<td>10 mm</td>
<td>7 mm</td>
<td>4 mm</td>
</tr>
<tr>
<td>\textit{Staphylococcus aureus}</td>
<td>12 mm</td>
<td>10 mm</td>
<td>5 mm</td>
<td>-</td>
<td>-</td>
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<tr>
<td>\textit{Pseudomonas}</td>
<td>7 mm</td>
<td>5 mm</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>\textit{Klebsiella}</td>
<td>9 mm</td>
<td>3 mm</td>
<td>-</td>
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BIBLIOGRAPHY


