INTRODUCTION

Medicinal plants have been contributing for the management of cancer since time immemorial. With a view to develop an eco-friendly herbal drug for cancer, many phytoconstituents and their derivatives were subjected to clinical and preclinical studies. Based on literature review it is observed that many of the Euphorbiaceae members such as Phyllanthus urinaria, Mallotus philippensis and Tragia plukenetti were screened for their anticancer potentials against various cancers and the results were encouraging this prompted us to select common Euphorbiaceous plant available in SASTRA campus for screening against EAC cell lines. Phyllanthus maderaspatensis (Euphorbiaceae) a common weed in SASTRA University campus. This taxon is an erect herb distributed in many places ranging from South Africa to Sri Lanka. Phyllanthus species are used since ancient times in different systems of medicine, particularly for treating liver disorders and urinary tract infections. Extract of aerial parts of Phyllanthus maderaspatensis are used as a hepatoprotective agent, also useful in headache, bronchitis, ear ache, ophthalma, gripping, cough, ascites, incipient, blindness, sores, ukers, stomachache, inflammations, intestinal spasms, gonorrhea, antimicrobial and viral infections. Traditional healers use this plant in treating fever and burns. The drug is reported for its chemoprotective, anti-edematous, anti-dysenterial, laxative, carminative, diuretic and immunomodulatory effects. P. maderaspatensis contains essential oil, madarin, mucilage, and β-sitosterol. Seeds of P. maderaspatensis contain long chain fatty acids and β-sitosterol. Defatted seed cake contains mucilage, which yields galactose, arabinose, rhamnose and aldobionic acid, niruriside, phyllanthin, hypophyllanthin and cinnamoyl sucrose acetate.

MATERIALS & METHODS

Plant Collection

Whole plant materials of P. maderaspatensis were collected from in and around SASTRA University campus, Thanjavur, Tamil Nadu, identified in the department of Centre for Advanced Research in Indian Systems of Medicine (CARISM), SASTRA University, Thanjavur, and authenticated with herbarium specimen of Rapinat Herbarium, St. Joseph’s College, Trichy. The plants were cleaned, shade dried and coarsely powdered.

Preparation of plant Extract

The course powdered plant material was extracted by cold maceration with n-hexane for 48 hours and then extracted using methanol for 48 hours. The extracts were concentrated under the vacuum in rotary evaporator and stored in an airtight container and refrigerated.

Preliminary phytochemical analysis

The n-hexane extract and methanol extracts were subjected to preliminary phytochemical screening as per standard textural procedure and results are tabulated.

HPTLC fingerprint analysis

HPTLC fingerprint analysis was carried out to identify lupeol and phyllanthin

Standard preparation:

Standards were prepared by dissolving 10 mg of lupeol and phyllanthin in 10 ml of chloroform and methanol respectively.

Sample preparation

100 mg of methanol extract of P. maderaspatensis diluted with 10 ml of MeOH and used for the identification of lupeol and phyllanthin.

Chromatographic parameters and conditions

TLC was run using mobile phases 1) hexane: ethyl acetate (8:2) for lupeol and 2) hexane: acetone: ethyl acetate (74:12:4) for phyllanthin.

In vitro cytotoxicity was carried out using Trypan blue exclusion method using various concentrations of the extracts (50, 100, 250, 500, 1000 µg/ml) and the % cytotoxicity was calculated using standard formula.

RESULTS AND DISCUSSIONS
Preliminary phytochemical analysis
The data of the preliminary phytochemical screening of hexane and methanol extracts were given in the Table No. 1 and noted the presence of flavonoid compounds, tannin, triterpenoids, carbohydrates and proteins in both extracts.

High Performance Thin Layer Chromatographic Finger Prints
In the present study a HPTLC method is reported to ensure the presence of lupeol content in various fractions of *Phyllanthus maderaspatensis*. Under the conditions the most suitable mobile phase consisted of hexane: ethyl acetate (8:2) which gave best resolution of free lupeol at Rf 0.54. Lupeol band observed, confirmed the presence of lupeol in the extract.

Phyllanthin was identified in methanol extract of *P. Maderaspatensis* in the mobile phase which consisted of hexane/ acetone/ ethyl acetate (74/12/8). The phyllanthin is visible in both reference and test solution tracks at (Rf 0.24).

Table 1: Preliminary phytochemical screening of hexane and methanol extracts of *P. maderaspatensis*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Test</th>
<th>Hexane extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>Wagner’s test</td>
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<td></td>
<td>Hager’s test</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Vitamin – C</td>
<td>Sodium nitro-pruside test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proteins and Amino acids</td>
<td>Millon’s test</td>
<td>-</td>
<td>+</td>
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<tr>
<td></td>
<td>Ninhydrin test</td>
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<td>+</td>
</tr>
<tr>
<td></td>
<td>Glycoside test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molisch test</td>
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<tr>
<td></td>
<td>Benedit’s test</td>
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<td>+</td>
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<tr>
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<td>Alkaline test</td>
<td>-</td>
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</tr>
<tr>
<td>Triterpenoids</td>
<td>Libermann-Buchard test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Gelatin test</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ indicates Positive. - indicates Negative.

Fig. 2: HPTLC – Lupeol

Fig. 3: Chromatogram for lupeol
**In Vitro Cytotoxic effect of methanol extract of against EAC cell lines using Trypan blue method**

Plant extract under study was tested for their cytotoxic potentials against Ehrlich Ascites Carcinoma cell lines using trypan blue method. The results revealed that methanolic plant extract possess cytotoxicity against EAC cell lines 93.62% of cell death was observed at 1000 µg/ml. The cytotoxicity effect of the methanolic plant extract was dose dependent. Maximum cytotoxicity effect was observed at the dose level 1000 µg/ml.

**Fig. 6: In vitro cytotoxic studies of P. maderaspatensis**

As a part of the apoptosis process there must be a considerable loss of membrane integrity and there by the cells will perm the dye to enter. In the present work the extract might have had considerable membrane damaging effect which might have permitted trypan blue to enter cancerous cells causing death of cancer cells.

**DISCUSSION**

Present study suggested that the test drug might have activated apoptosis. Presence of hepatoprotective molecule phyllanthin is very interesting feature observed in the present study. This molecule can help not only in reverting back the hepatic marker enzymes such as AST, ALT, ALP and GGT but also can contribute towards the inhibition of tissue necrosis, organ destruction and cell injury. Altered metabolic conditions are often encountered in malignancy due to these abnormal variations in liver marker enzymes, which can be corrected by the presence of phyllanthin in the extract. Flavone like lupeol is a proven antioxidant and can help in providing enhanced antioxidant mechanism and contribute towards the prevention of cancer cell growth.15,16

**CONCLUSION**

Preliminary phytochemical analysis was carried out to find out the major chemical constituents present in the *P. maderaspatensis* the drug under study. Methanol extract contained tannins, triterpenoids, flavonoids, proteins and carbohydrates. Trypan blue method was employed to assess the cytotoxic potential of the plant extract under study. The results of *in vitro* cytotoxicity study depicted that the methanolic extract of *Phyllanthus maderaspatensis* possess good cytotoxic potentials at higher concentration (93.62% of inhibition observed in 1000 µg/ml). The activity might be due to the presence of secondary metabolites such as flavone and phyllanthin present in the plant extract. Flavonoids are known to implicate signal transduction in cell proliferation and thereby prevent cancer. The preliminary data obtained in the present work suggested that further in depth studies can result in the development of a novel new non-toxic anticancer herbal drug from this traditional source.

**ACKNOWLEDGMENT**

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