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Cytotoxicity Assessment, Photochemical and FTIR Analysis of *Euphorbia hirta* – *in Vitro* Study

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ABSTRACT
Cancer may reveal different aspects of disease characteristics in different data modalities and thus integrating imaging with non-imaging biomarkers and clinical data has the potential to discover information that is not obvious in any single data modality. We are developing methods for integrating image, genomic and clinical data to improve precision medicine applications for cancer patients. Our results suggest that the integration strategies achieve improved prediction results than using each single data modality alone. We further show that information is transferrable across age groups or tumour subtypes under the data fusion framework.
Keywords: *Cytotoxicity Assessment, Photochemical Analysis, FTIR Analysis, Euphorbia Hirta, In Vitro Study, UV-VIS Analysis.*

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INTRODUCTION

Cancer is a major public health problem, with significant associated death and disability. It is the second leading cause of death in developed countries and is one of the three leading causes of death for adults in developing countries. Of the 12.4 million new cancer cases in 2008, the most common cancers in terms of incidence were lung (1.52 million), breast (1.29 million) and colorectal (1.15 million) [2].Cancer chemoprevention is defined as the use of natural, synthetic, or biological or chemical agent to reverse, suppress, or prevent carcinogenic progressive to invasive cancer. Several studies reported that medicinal plants and their products have shown to chemoprevention and chemotherapeutic effects in animal models.

Plant phytochemicals on cancer

Plant materials were used for the treatment of malignant diseases for centuries. Recent phytochemical examination of plants which have a suitable history of use in folklore for the treatment of cancer had induced often resulted in the isolation of principles with anti-tumors activity. In this Study as using the Experimental plant *Euphorbia hirta.*

MATERIAL AND METHODS

Collection of Plant Materials

The fresh leaves of *Euphorbia hirta*was collected from herbal garden, St. Joseph College Tiruchirappalli, Tamilnadu.

Preparation of Seed and Leaf Powder

The leaves of *Euphorbia hirta*was washed with sterile distilled water thrice, cut into small pieces and shade dried at room temperature for two weeks and made into a coarse powder using mechanical blender and stored in an airtight container.

Extraction of Plant Material

Materials

Sample	:	The leaves of *Euphorbia hirta*
Solvents	:	Ethanol and aqueous.
Apparatus used	:	Soxhlet apparatus

Procedure

The powder of leaves of *Euphorbia hirta* was successively extracted with ethanol and aqueous by the successive solvent extraction method using a Soxhlet apparatus according to the methodology of Indian Pharmacopoeia [2]. The extraction was carried out for 72 hours with the selected solvents with a ratio 1:4 w/v, based on their polarity *viz*., ethanol and aqueous.

Phytochemical Studies

The ethanol and aqueous extracts of leaves, obtained by successive solvent extraction were subjected to various phytochemical analyses to detect the phytoconstituents present in them.

Test for Alkaloids

1 g of leaf powder was extracted with 20 mL alcohol by refluxing for 15 minutes, filtered and the filtrate was evaporated to dryness. The residues were dissolved in 15 mL of sulphuric acid (H_2S0_4) (2N) and filtered. After making it alkaline, the filtrate was extracted with chloroform. The residue left after evaporation was tested for the presence of alkaloids with Dragondroff's reagent. Development of orange coloured precipitate indicated the presence of alkaloid.

Tests for Saponins Foam test-

Each extract (50 mg) was diluted with distilled water and made up to 20 mL. The suspension was shaken in a graduated cylinder for 15 minutes. A 2 cm layer of foam indicated the presence of saponins.

Tests for Tannins

Reaction with lead acetate

To the extracts of the plant, 2 mL of 10% w/w solution of lead acetate was added. Formation of precipitate indicated the presence of tannins.

Tests for Phenolic Compounds

Ferric chloride ($FeCl_3$) test [7]

1ml of extract was separately shake-n with water and warmed. Now about 2 mL of 5% $FeCl_3$ solution was added and observed in the formation of green or blue colour which indicated the presence of phenolic compounds.

Test for Flavonoids - Shinoda Test

1 g of powder was extracted with 10 mL of solvents for 15 minutes in a boiling water bath and filtered. To the filtrate, a small piece of magnesium ribbon and 3 to 4 drops of concentrated H_2S0_4 were added. Red colour formation indicated the Presence Of Flavonoids

Test for Steroids-Leibermann-Barchard Test

Powder was dissolved in 2 ml of chloroform in a dry test tube. 10 drops of acetic anhydride and two drops of concentrated H_2S0_4 were added. The solution becomes red, then blue and finally bluish colour formed, which indicated the presence of steroids.

Tests for Triterpenoids

4 mg of each extract was added to 0.5 ml of acetic anhydride, 0.5 ml of chloroform, 0.5 ml of concentrated H_2S0_4 was added slowly and red violet colour was observed for the presence of terpenoids.

Tests for CoumarinsWith sodium chloride (NaCl**)**

0.5ml of extract was treated with 1 ml of 10% NaCl and observed for the formation of yellow colour which indicates the presence of coumarins.

Tests for Glycoside - Borntrager's Test

Legal's test

To 2 ml of extract, 3 ml of chloroform was added and shaken well, chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicated the presence of glycosides.

Test for Quinine

To 1 ml of extract, 2 ml of 5% KOH was added. Change in colour was observed. Pink colour showed the presence of quinone.

Test for Volatile Oils

To 2 ml of extract 0.1 ml diluted NaOH and 0.1 ml of diluted HCL was added. Formation of white precipitate indicated the presence of volatile oil.

Cell Line Culture

Cell Culture

The cancer cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA), while Ehrlich Ascites carcinoma cell line was purchased from the Health Protection Agency (HPA) Culture Collection (Salisbury, England, UK). The cells were maintained 15% -Fetal Bovine Serum and incubated at 37 ˚C in a humidified atmosphere, 5% CO2 atmosphere.

Test Sample Preparation:A stock solution of 30 mg/mL of the tested fraction was prepared by dissolving it with DMSO. Subsequently, working solution of different concentrations was prepared by

dissolving the stock solution with culture medium. DMSO only (0.5%, *v/v*) was used as the vehicle control in complete culture medium.

Cytotoxicity assay

Cellular cytotoxicity was determined by the MTT reduction assay. This Colorimetric assay is based on the capacity of mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4,5-dimethyl 2 thiazolyl)-2,5-diphenyltetrazolium brom-ide (MTT) into an insoluble, coloredformazan product which is measured spectrophotometrically [17]. Growing concentrations of the tested extract 5, 10, 20, 25(µg/mL) were applied to the wells of a 96-well plate containing the confluent cell monolayer (106 cells per well) in duplicate. Methotrexate as positive control drug was added in the same concentrations and conditions. After 48 hours of incubation at 37˚C, 20 µL of the MTT solution [5 mg/mL in Phosphate buffered saline (PBS)] was added. After incubation in the same conditions for 4 hours, the plates were treated with a mixture of HCl / Isopropanol (24:1) to dissolve the blue intracellular formazan product. One hour later, the plates were read on a Micro ELISA reader using two wavelengths (570 and 630 nm). DMSO was used as negative control. The optical density (OD) of each well was measured at 550 nm by using a Synergy microplate reader. The percentage of cytotoxicity compared to the untreated cells was determined. -

The Percentage o-f cytotoxity was calculated as Follows

$$\text{\% of Cytotoxity} = - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

Table 1 Phytochemical analysis of leaf extract of *Euphorbia hirta*

S.No	Phytochemical	Aqueous extract	Ethanolic extract
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Steroids	+	+
4	Tannins	+	+
5	Terpenoids	-	+
6	Quinine	+	+
7	Coumarins	-	+
8	Starch	+	+
9	Saponins	+	+
10	Phenols	-	+
11	Protein	+	+

Table 2 Thin Layer Chromatography of*Euphorbia Hirta L* Extracts

S.No	Extracts	Spot	Rf Value
1	Crude water	Brown Spot	0.50
2	Crude alcohol	Green Spot	0.83-
3	Soxhlet water	Brown Spot	0.45
4	Soxhlet alcohol	Yellow Spot	0.56

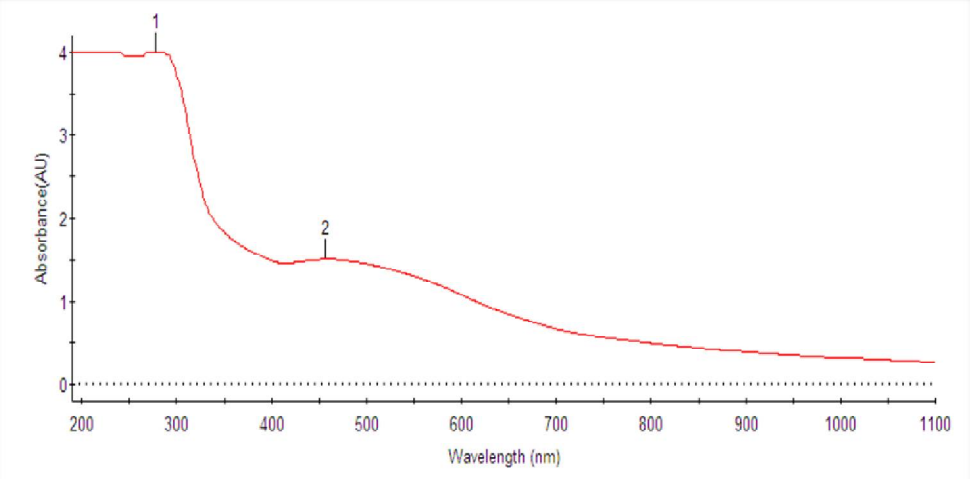

Fig 1: UV-VIS Analysis of *Euphorbia hirta*

Table 3 UV-VIS Analysis of *Euphorbia hirta*

S.No	Wave Length	Absorbance
1	278.00	4.0000
2	457.10	1.5088

DISUSSION

Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active phytoconstituents of therapeutic value.he most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Many of these indigenous medicinal plants are used as spices. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes **[**9,10]According to [11] the flavonoids have long been recognized to possess antiallergic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic activities as well as to affect some aspects of mammalian metabolism. TLC of plant extract in choloroform reports three spots for various phytochemicals. The reported spots are separated with enough space and having various R_f values showing the presence of atleast three phytochemicals in ethanol extracts Absorption bands observed pertaining to *Euphorbia hirta*plant extract are displayed in figure 2.In the UV-VIS spectra the appearance of one or more peaks in the region from 200 to 400 nm is a clear indication of the presence of unsaturated groups and heteroatoms such as S, N, O . The spectrum for *Euphorbia hirta*shows two peaks at positions 278 nm, and 457 nm. This confirms the presence of organic chromophores within the *Euphorbia hirta*extract. Nevertheless, the use of UV-visible spectrophotometery in the analysis of complex media is limited by the inherent difficulties in assigning the absorption peaks to any particular constituents in the system. This one of the methods to assess cytotoxicity of anticancer drugs. The test is based on the principles that living cell membrane has the ability to prevent the entry of dye and can be easily distinguished from dead cells which takes the dye. This preliminary experiment was carried out using four different concentrations of the plant extracts and its isolated fraction under study.

In addition, many of these treatments present limited anti-cancer activity [8] Therefore, there is a need to discover alternative anticancer drugs, hopefully more potent, as well as more selective and less toxic than those currently in use.

Screenings of medicinal plants used as anticancer remedies has provided modern medicine with effective cytotoxic pharmaceuticals. More than 60% of the approved anticancer drugs in United State of America (from 1983 to 1994) were from natural origin [5]. In this last decade, investigations on natural compounds have been particularly successful in the field of anti-proliferative drug research.

In addition, cytotoxic assays do not provide false negative results since they consider Initial scientific investigations of fig latex against cancer were performed in the 1940s [4]. Injection of an extract of *Ficuscarica*latex was found to inhibit growth of a benz-[*a*]-pyrene-induced sarcoma and resulted in the disappearance of small tumours in albino rats. This work inspired the isolation and structure elucidation of a mixture of 6-*O*-acyl-β-d-glucosyl-β-sitosterol isoforms from the latex of *Ficuscarica*that demonstrated anti-proliferative activity in several tumour cell lines [15].

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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