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Anticancer Activity of *Pedalium Murex.L*(Flowers) Against Human Liver Cancer(Hepg2) Cell Line

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Abstract : Objective : To investigate the anticancer activity of the flower *of Pedalium murex. L* against human hepatoma cell line (HepG2).
Methods: In vitro anticancer activity was carried out to screen cytotoxicity potency of the solid obtained from ethyl acetate fraction of *Pedalium murex.L* flower extract at different concentrations against HepG2 cell line. The MTT (methylthiazolyldiphenyl- tetrazolium bromide) assay for cell viability and markers is predictable to confirm the cytotoxicity.
Result: The solid obtained from ethyl acetate fraction from the flower extract of *Pedalium murex. L* was tested for its anticancer activity against HepG2 cell lines (liver cancer) at various concentrations by MTT assay. It was confirmed that the IC_{50} value of this sample was 144 ± 5.3 µg/ml against Liver Cancer HepG2 cell line.
Conclusions: *Pedalium murex. L* is a potential plant with anticancer activity. The isolation of the pure compounds and determination of the bioactivity of individual compounds will be further performed.
Keywords: Pedalium murex. L; anti-cancer activity; MTT assay; HepG2; cytotoxicity.

Introduction

Cancer is a standout amongst the most extreme illness and a noteworthy general medical issue in many parts of the nation. Cancer keeps on remaining for the significant reason for passing's on the planet and claims more than 6 million lives each year [1].

The International Agency for Research on Cancer (IARC) gauges the rate of mortality and pervasiveness from significant sorts of cancer cell, at national level. For 184 nations of the world uncovered that there were 14.1 million new cancer cases, 8.2 million cancer passing's and 32.6 million individuals were living with cancer in 2012 around the world. By 2030, it is anticipated that there will be 26 million new cancer cases and 17 million cancer passing's for every year [2-5].Primary liver cancer (PLC) has two major form of Hepatocelluar carcinoma (HCC) and intrahepatic cholangiocarcinoma(ICC), accounting for approximately 90% and 5% respectively(6-7).HCC causes highannual mortality rates, particularly in Thailand, Cambodiaand Laos, where viral hepatitis is endemic[8]. The induction of HCC is preceded by the occurrence of hepatocellular damage via reactive oxygen species (ROS) and the generation of chronic inflammation related to hepatocarcinogenesis[9].Notwithstanding, the fundamental issue of chemotherapy to treat HCC is the tumor resistance system, due to up control of the multi-tranquilize resistance protein (MDR) and a decline of apoptotic

proteins [10].Subsequently, more powerful Chemotherapy is expected to control cancer and apoptosis acceptance, which is the coveted impact for effective cancer treatment [11].

Pedalium murex.L is a member of sesame family, Pedaliaceae. It is found in different part of the world such as tropical area, Srilanka, India, Mexico and Pakistan . In India , it occurs mainly in the Western and Corommandal coasts as weed of waste places and is generally called under the Hindi name ‘’ Gokhru or gokhar’’ and in Sanskrit as ‘’gaja-daunstraka, gokshura or titta –gokshura”. Its name vary from one region to another ranging from North to South and from East to West part of the country. Its also called in

Kannada (doddaneggilu), Malayalam (motha-malvi-gokharu), Tamil (Yanainerunjil &Ananerinnil), Marathi (Gokhara),Gujarati (Gokhura), Oriya(Yanainerunjil) ,Arabic (Khasakekabir) and Singapore (Atineranchi)[12-14]. An infusion or extract prepared from the different part of the plant in cold water is used as demulcent, diuretic , and also found to be best in the treatment of disorders of urinary systems such as gonorrhoea, dysuria, incontinence of the urine and vice versa [15-16].This plant is also used by the local people as analgesic and antipyretic activities [17-18].So there is a need to assess the potential effects of this plant. Keeping this in view, the present study has been undertaken to investigate the anticancer potential of the solid obtained from the ethyl acetate fraction from the flower *Pedalium murex .L.*

Materials and Methods

Collection of flowers

The fresh flowers of *Pedalium murex.L* were collected from Z. Suthamalli, Ariyalur (Dt), Tamil Nadu, India, during the month of January and identified by Dr.S.John Britto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. DP002 dated: 22/01/2016). St.Joseph’s College (Campus), Trichirappalli, Tamil Nadu, India.

Extraction and fractionation

Fresh flowers (3 kg) of *Pedalium murex.L* were extracted with 90% ethanol (5x500ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with petroleum ether (60-80^{0}C) (6x250ml), Peroxide free diethyl ether (4x250ml) and ethyl acetate (8x250ml). Petroleum ether fraction and diethyl ether fraction did not yield any isolable material. Ethyl acetate fraction on concentration yielded a dry powder which was dissolved in DMSO to get various concentrations and were used for further study.

In vitro Anti-cancer Activity

Cell line and culture

HepG2 (liver) cell lines were obtained from National Center for Cell Sciences Pune (NCCS). The cells were maintained in Minimal Essential Media (MEM) supplemented with 10% FBS (Foetal Bovine Serum), penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml of CO_2 at 37^{0}C.

Reagents

MEM was purchased from Hi Media Laboratories, FBS was purchased from Cistron laboratories, Trypsin, methylthiazolyldiphenyl- tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO) were purchased from Sisco research laboratory chemicals, Mumbai. All the other chemicals and reagents were obtained from Sigma Aldrich, Mumbai.

Principle of MTT assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay was the first homogeneous cell viability assay developed for a 96-well format that was suitable for high throughput screening (HTS). The MTT tetrazolium assay technology has been widely adopted and remains popular in academic labs as evidenced by thousands of published articles. The MTT substrate is prepared in a physiologically balanced solution, added to cells in culture, usually at a final concentration of 0.2 - 0.5mg/ml,

and incubated for 1 to 4 hours. The quantity of formazan (Directly proportional to the number of viable cells) is measured by recording changes in absorbance at 570 nm using a plate reading spectrophotometer.

Viable cells with active metabolism convert MTT into a purple colored formazan product with an absorbance maximum near 570 nm . When cells die, they lose the ability to convert MTT into formazan, thus color formation serves as a useful and convenient marker of the viable cells. The exact cellular mechanism of MTT reduction into formazan is not well understood, but likely involves reaction with NADH (The redox reactions of nicotinamide adenine dinucleotide) or similar reducing molecules that transfer electrons to MTT. Speculation in the early literature involving specific mitochondrial enzymes has led to the assumption mentioned in numerous publications that MTT is measuring mitochondrial activity.

The formazan product of the MTT tetrazolium accumulates as an insoluble precipitate inside the cells as well as being deposited near the cell surface and in the culture medium. The formazan must be solubilized prior to recording absorbance readings. A variety of methods have been used to solubilize the formazan product, stabilize the color, avoid evaporation, and reduce interference by phenol red and other culture medium components. Various solubilization methods include using: acidified isopropanol, DMSO, dimethylformamide, SDS (Sodium dodecyl sulphate) and combinations of detergent and organic solvent. Acidification of the solubilizing solution has the benefit of changing the color of phenol red to yellow color that may have less interference with absorbance readings. The pH of the solubilization solution can be adjusted to provide maximum absorbance if sensitivity is an issue; however, other assay technologies offer much greater sensitivity than MTT.

In vitro assay for cytotoxicity activity(MTT assay)

The cytotoxicity of sample (*Pedalium murex.L*) on HepG2 (liver) cell line was determined by the MTT assay. Cells (1 × 105/well) were plated in 1ml of medium/well in 24-well plates (Costar Corning, Rochester, NY). After 24 hours incubation, the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 24 h at 37^0C. After removal of the sample solution and washing with phosphate- buffered saline (pH 7.4), 200µl/well (5 mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl- tetrazolium bromide (MTT) phosphate -buffered saline solution was added to cells. After 4 h incubation, 100µl Solubilization solution(pH 4.7 for 40%(v/v)DME in 2%(v/v)glacial acetic acid and 16%(wt/v)SDS) were added. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically. The absorbance at 570 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of HepG2 was expressed as the % cell viability, using the following formula:

% Cell viability = (A_{570} of treated cells / A_{570} of control cells)*100

Statistical analysis

All the data were reported as the mean ± standard deviation (S.D.). All statisticalanalysis was performed by means of one-way analysis of variance (ANOVA) andStudent's *t*-test using Graph Pad Prism statistical software package version7.02. Only a value of $p < 0.05$ and 0.01 was considered statistically significant.

Results

In vitro assays (Cytotoxic studies):

The anticancer activity of the*Pedalium murex.L* flower was confirmed by MTT assay (Table 1, Fig 1&2). The control HepG2 cells showed high proliferation that has been taken as 100%. Test samples showed effectiveIC_{50} value against HepG2 (liver) cell line was found to be 144 ± 5.3µg/ml. It induced cytotoxicity in a significant manner which implicit the damage to the membrane integrity of the cell when compared with control. The cytotoxicity was increased with increase in concentration of the sample and near normal level was attained at various concentrations (10µg/ml, 25µg/ml, 50µg/ml, 100 µg/ml and 250µg/ml) and maximum effect was found when treated at 250µg/ml, which showed cell viability of 43.42± 0.8% . From the above results, it

was confirmed that the solid obtained from ethyl acetate fraction of *Pedalium murex* .*L*extract at 250µg/ml seems to offer significant protection and maintains the structural integrity of the hepatocellular membrane.

Table 1:Cell viability (%) of HepG2 cell line

Concentration (µg/ml)	**Cell Viability (%)**	IC_{50} **(µg/ml)**
Control	100	144 ± 5.3
10	83.58 ± 0.8**	
25	74.33 ± 1.0**	
50	64.42 ± 0.8**	
100	53.67 ± 1.0**	
250	43.42 ± 0.8**	

Values shown as for means±S.D. of triplicate.
**** *p*< 0.05 & 0.01 compared with control (one-way ANOVA and *t*-test).**

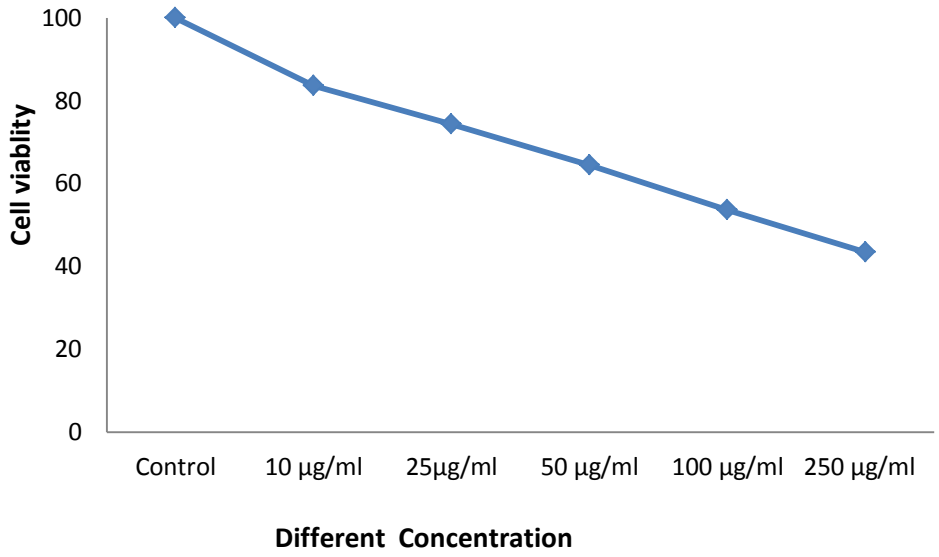

Fig. 1: Graphical representation of the cell viability (%) values of the solid obtained from the ethyl acetate fraction of *Pedalium murex.L*(flowers)against HepG2 cell line.

Discussion

Ominous free radicals are created in the body amid typical digestion and furthermore upon presentation to ecological poisons, for example, irresistible operator, UV light, radiation et cetera. Unsafe free radicals are not killed by the body's essential and auxiliary safeguard system on abundance of horrible free radicals [19]. Existing clinical reviews have likewise demonstrated that supplemental levels of anti-oxidant vitamins (E, C and B complex) diminish, the individual hazard for certain malignancy [19, 20, 21, 22]. Many of the medicinal plants have been found to be effective in experimental and clinical cases of cancer. Medical plants dominate immunomodulatory and anti-oxidant properties, leading to anti-cancer activity [19, 23, 24, 25]. This plant has been reported to be responsible for free radical scavenging effects and anti-oxidant property [26-29]. MTT is considered to be reliable assay to determine the extent of cell viability. In the present study, The cell treated with the test sample at various concentration (10, 25, 50,100 and 250µg/ml) showed the protective nature of the sample against the deleterious effects and the maximum effect was observed at 250µg/ml.The fraction had an IC_{50} value of 144 ± 5.3µg/ml which assumed cell viability. From the results,it is clear that the solid obtained from the ethyl acetate fraction of *Pedalium murex .L* flower has cytotoxic effect against HepG2 cell line.

MTT Assay on HepG2 Cell line (flowers extract)

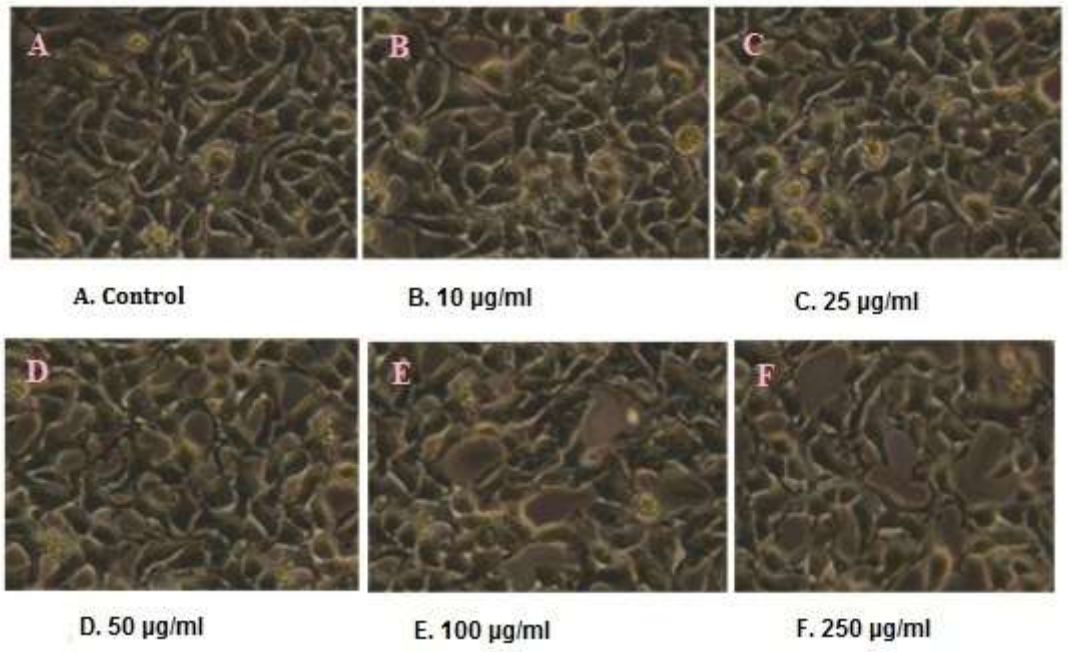

Fig.2 :(B-F) Effect of the solid obtained from the ethyl acetate fraction of *Pedalium murex.L* flowersagainst human Liver cancer HepG2 Cell line in differentconcentrations.

Conclusion

The results obtained from the *in vitro* studies performed using the HepG2 cell lines reveals that the solid obtained from theethyl acetate fraction of the EtOH extract of

Pedalium murex .L flowers has a moderate anticancer activity even though cell growth inhibition were increased when concentration of sample was increased.These concentrations were able to induce apoptosis on human cancer cell lines and its anticancer activity was found to be precise. Further work is required in order to establish the identity of the chemical constituent responsible for anticancer activity. Studies are in progress in our laboratory to elucidate the molecular structure of the compound. This contributes towards the development of potent anticancer drug.

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