The characteristic of plants from kerala

Environment, Plants

Material and methods

Dried seeds of vitis viniferae, flowers of Ixora coccinea and roots of piper longum were collected in the month of may from Kerala. Morphological and microscopical characters of the plant was first identified with description given in the different literature review(11, 12, 13). Later plants were authenticated by Prof. M. D. Rajanna at Botonical garden , University of agricultural sciences, GKVK, Bangalore, Karnataka. All the plant parts were shade dried and reduced to powder separately and stored at room temperature in airtight containers. All 3 plant parts were extracted by ethanol separately.

Method(14, 15): MTT Powder [3-{4, 5-dimethylthiazol-2-yl}-2, 5-diphenyltetrazolium bromide], CO2 incubator, 70 % Ethanol, DMEM(Dulbecco's Modified Eagle's Medium), Microplate reader (Tecan). Cell lines and culture medium. Stock cells was cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), 1% penicillin (100 IU/ml), and streptomycin (100 µg/ml) in an humidified atmosphere of 5% CO2 at 37 oC. The cell was dissociated with TPVG (Trypsin Phosphate Versene Glucose)solution (0. 2 % trypsin, 0. 02 % EDTA, 0. 05 % glucose in PBS). The viability of the cells are checked using trypan blue(dye) and centrifuged. Further, 50, 000 cells / well of L929((mouse fibroblast cell line: Adherent cells) was seeded in a 96 well plate and incubated for 24 hrs at 37oC, 5 % CO2 incubator.

Cell line: Human breast adenocarcinoma MCF 7 cell line was procured from ATCC(American Type Culture Collection).

Cell proliferation by MTT assay(16, 17): Test samples were placed in each well of the 96 well microtiter culture plate. The L929 monolayer cells were trypsinized and the cell count was adjusted to 5. 0 x 105 cells/ml using DMEM containing 10% FBS.

To each well of the 96 well microtiter plate, 100 µl of the diluted cell suspension (50, 000 cells/well) was seeded on each scaffold and cells seeded on cell culture plate. The plates were then incubated at 37oC for 1 day in 5% CO2 atmosphere. After 24 h, the test solutions in the wells were discarded and 100 µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were gently shaken and incubated for 4 h at 37o C in 5% CO2 atmosphere. The mitochondrial dehydrogenase enzymes of viable cells cleave the tetrazolium ring to an insoluble purple formazan. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the intracellular formed formazan and the absorbance was measured using a microplate reader at a wavelength of 590 nm. percentage inhibition was calculated using formula, Percent inhibition =(OD of control-OD of sample)×100.