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PHYTOCHEMICAL SCREENING AND *IN VITRO* EVALUATION OF CYTOTOXIC ACTIVITY OF FRUITS OF MEYNA SPINOSA ROXB

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ABSTRACT

Meyna spinosa Roxb, a medicinal plant enjoys it use in the traditional medicine in all over the India for the treatment of a number of ailments. In Bengali it is called as Monkata. It is a wild common plant distributed in India, Bangladesh and in both northeastern tropical Africa and tropical Asia. The main objective of the present research work was to determine various bioactive compounds especially phenolic and flavonoid contents, carbohydrates, phytosterols, aminoacids and proteins and to evaluate the *in vitro* cytotoxic activity of different fruit extracts of Meyna spinosa Roxb . Based on this, a new series of constituents had been planned to extract by Methanol (ML), Ethanol (EL), and Chloroform (CF) from the fruits of *Meyna spinosa Roxb*. The *in-vitro* cytotoxic activity was carried out against Human Prostate cancer cell line DU-145 and SRB assay was used to analyze the cell growth inhibition of the cancer cell line. 5-Flurouracil (5-FU, at 10µg/ml) was used as a standard drug for DU-145 cell line. The results showed that the various extracts of fruits of *Meyna spinosa Roxb*, had a potential cytotoxic activity against DU-145 cell lines. The IC_{50} values of the various extracts of fruits of *Meyna spinosa Roxb* were found to be ML (IC_{50} of 2.5 µg/ml), EL (IC_{50} of 2.3 µg/ml), and CF (IC_{50} of 2.1 µg/ml) for DU-145 cell line.

INTRODUCTION

MATERIALS AND METHODS

Chemicals and drugs

The all chemicals used for the extraction and phytochemical screening were of LR and AR grade. The standard drug 5-FU was purchased from local retail pharmacy.

Apparatus and chemicals required

Round bottom flask, water condenser, heating mantle, motor and pestle, methanol, ethanol, chloroform, dichloromethane, sodium chloride solution, magnesium sulfate etc.

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Extraction

Weigh 50 g of fruits of *Meyna spinosa Roxb* (ripen can be mashed to prepare a paste) into a 500 ml round-bottomed flask. Add 200 ml of methanol and 240 ml of dichloromethane. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separatory funnel. Wash this mixture containing bioactive compounds with three portions of 250 ml each with sodium chloride solution. Dry the organic layer over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating (Raj K. Bansal, Jan 1, 2009). The same procedure has been followed for the preparation of EL and CF extracts.

Preliminary Phytochemical screening (Satyanarayana and Chakrapani, Dec 1, 2013; Dandiya and Sharma, 2004; Chatterjea and Rana Shinde, 2008; Kokate *et al*., Nov 29, 2012; Jaswant Kaur, 2010; Devala Rao, 2006 and Gurdeep R. Chatwal, 2006) Preliminary phytochemical screening of various extracts (ML, EL and CF) of fruits of *Meyna spinosa*

Roxb had shown the presence of following bioactive compounds which were confirmed by their specific qualitative confirmatory chemical tests: Proteins and amino acids, Carbohydrates, Glycosides, Alkaloids, Terpenoids, Saponins, Phytosterols, Flavanoids and phenolic compounds, Gum and mucilage etc.

Screening of *invitro* cytotoxic activity by SRB assay

Principle

Sulphorodamine B (SRB) is a bright pink aminoxanthine dye with two sulfonic acid group. Under mild acidic conditions SRB dye binds to basic amino acid residues in trichloro acetic acid (TCA) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of visible at least two order of magnitude (Skehan *et al*., 1989 and Skehan *et al*., 1990)

Cell culture

Human Prostate cancer cell line DU-145 was provided by National Centre for Cell Science (NCCS), Pune and were grown in Eagles Minimum Essential Medium (EMEM) which contained 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 100% relative humidity, 5% CO2, 95% air and the culture medium was changed twice a week.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 0.5-1.0x10^5 cells/ml using medium containing 10% new born sheep serum. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately) 10,000 cells was added. After 24 hrs, when a partial monolayer was formed, the supernatant was flicked off, washed once and100 µl, 50µl and 25µl of different concentration of extracts of fruits of *Meyna spinosa Roxb* were added to the cell in microtitre plate. The plates were incubated at 37^{0}c for 72 hrs in 5% CO_2 incubator, microscopic examination was carried out and observations were recorded every 24 hrs.

After 72 hrs, 25µl of 50% TCA was added to wells gently such that it forms a thin layer over the test extracts to form overall concentrations 10%. The plates were incubated at 4^{0}c for 1 hr. The plates were flicked and washed five times with tap water to remove traces of medium sample and serum and were then air dried. The air dried plates were stained with 100 µl SRB and kept for 30 mnts at room temperature. The unbound dye was removed by rapidly washing four times with 1% acetic acid. The plates were then air dried. 100 µl of 10 mM Tris base was then added to the wells to solubilise the dye (Master, 2000). The plates were shaken vigorously for 5 mnts. The absorbance was measured using microplate reader at a 540 nm. The % growth inhibition was calculated by the following formula:

% cell growth inhibition = 100-{(At-Ab/Ac-Ab)}x 100
At = Absorbance value of test compound
Ab = Absorbance value of blank
Ac = Absorbance value of control

RESULTS AND DISCUSSION

The results for cell growth inhibition by the various extracts (ML, EL, CF) of fruits of *Meyna spinosa Roxb* against DU-145 cell line for various concentrations were shown in table 1, 2 and 3.

Table 1. For percentage (%) of cell Growth Inhibition of Methanolic Extract (ML) of Fruits of *Meyna spinosa Roxb* on DU-145 Cell lines by SRB Assay

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	25 µg/ml	0.022	92.54
2	50 µg/ml	0.028	90.50
3	100 µg/ml	0.035	88.13
4	10 µg/ml (5-FU)	0.0102	96.55
5	Control	0.295	0

Table 2. For percentage (%) of cell Growth Inhibition of Ethanolic Extract (EL) of Fruits of *Meyna spinosa Roxb* on DU-145 Cell lines by SRB Assay

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	25 µg/ml	0.019	93.56
2	50 µg/ml	0.030	89.83
3	100µg/ml	0.08	72.89
4	10 µg/ml (5-FU)	0.0102	96.55
5	Control	0.295	0

Table 3. For percentage (%) of cell Growth Inhibition of Chloroform Extract (CF) of Fruits of *Meyna spinosa Roxb* on DU-145 Cell lines by SRB Assay

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	25 µg/ml	0.017	94.23
2	50 µg/ml	0.022	92.54
3	100 µg/ml	0.035	88.13
4	10µg/ml (5-FU)	0.0102	96.55
5	Control	0.295	0

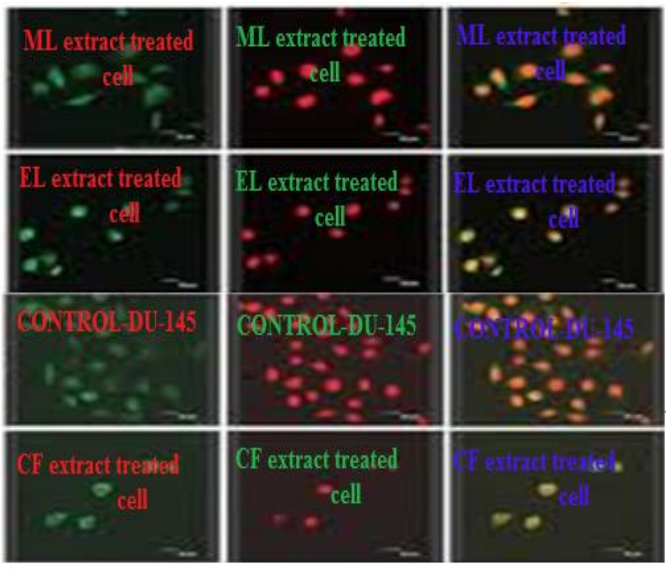

Fig. 1. Cytotoxic activity of various extracts of Fruit of *Meyna spinosa Roxb* against DU-145 Cell lines for % growth inhibition.

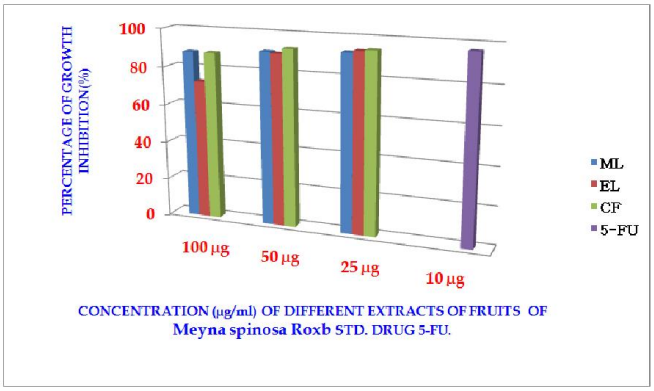

Fig. 2. Graphical representation of Cytotoxic activity of various extracts of Fruit of *Meyna spinosa Roxb* against DU-145 Cell lines for % growth inhibition

As the concentration increases there was an increased in the cell growth inhibition and it was found that all the extracts (ML, EL and CF) possessed potential cytotoxic activity against DU-145 cell lines. The IC_{50} values of the various extracts of fruits of *Meyna spinosa Roxb* were found to be ML (IC_{50} of 2.5 µg/ml), EL (IC_{50} of 2.3 µg/ml) and CF (IC_{50} of 2.1 µg/ml) for DU-145 cell line were more than 100 µg/ml and the regression values were difficult to analyze.

Conclusion

In conclusion, we report here that the various extracts of fruits of *Meyna spinosa Roxb* had the ability to kill tumour cells *in vitro*. The cytotoxic activities of the extracts ML, EL and CF against DU-145 cell lines can be considered very good with regards to the USNCI standard. It was also displayed that among these three extracts CF extract was found to be possess the highest cytotoxic activity with growth of inhibition 94.23% for DU-145 at the highest concentration 25 µg/ml, then EL extract with growth of inhibition 93.56% for DU-145 and lastly ML extract with growth of inhibition 92.54% for DU-145 cell lines at the highest concentration 25 µg/ml.

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