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EVALUATION OF *IN VITRO* ANTICANCER ACTIVITY OF ETHANOLIC EXTRACT OF CASTOR SEEDS (EE-CS) AGAINST HUMAN PROSTATE CANCER CELL LINE DU-145

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ABSTRACT

DU145 (DU-145) and PC3 human prostate cancer cell lines are the "classical" cell lines of prostatic cancer. DU145 cells have moderate metastatic potential compared to PC3 cells which have high metastatic potential. The DU145 cell line was derived from brain metastasis. DU145 are not hormone-sensitive and do not express prostate-specific antigen (PSA). It has been demonstrated that administration of NFkappaB ligand RANKL promoted DU145 cell invasion in bone, resulting in osteolytic lesions. DU145 cells also produce soluble factors that activate pre-osteoblast precursors and increase RANKL expression, thus facilitating prostate cancer metastasis in bone. The main objective of the present research work was the evaluation of *in vitro* anticancer activity of EE-CS (*Ricinus communis*). The *in vitro* anticancer activity was carried out against human prostate cancer cell line DU-145 by SRB assay. The results obtained from the *in-vitro* studies performed by SRB assay by using human prostate cancer cell line DU-145 displayed that EE-CS possessed good anticancer activity. From the present studied it had been concluded that EE-CS exhibiting the potential anticancer activity on DU-145 cell line which was proved by using standard drug 5-FU and it was found that EE-CS, with the highest 92.80% growth inhibition at 40 µg (IC_{50} = 2.5 µg/ml), with the 90.06% growth inhibition at 30 µg (IC_{50} = 2.9 µg/ml). The IC_{50} value of standard drug 5-FU was found to be 1.5 µg/ml with 96.54 % growth inhibition at concentration 50 µg/ml.

KEYWORDS

DU-145, Metastatic potential, Prostate-specific antigen, SRB, Assay and IC_{50} etc.

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INTRODUCTION

It is truth that without nature human being life is not possible. The food, clothes and shelter are three basic necessity of human beings and an important one necessity is good health, which provided by plant kingdom. Plant kingdoms are the rich source of organic compounds, many of which have been used for medicinal purposes. In traditional

medicine, there are many natural crude drugs that have the potential to treat many disease and disorders one of them is *Ricinus communis*; Family: Euphorbiaceae popularly known as 'castor plant' and commonly known as 'palm of Christ', Jada (Oriya), Verenda (Bengali), Endi (Hindi), Errandi (Marathi), Diveli (Guajarati)[1]. The plant is widespread throughout tropical regions as ornamental plants.

The castor oil plant is a fast-growing, suckering perennial shrub or occasionally a soft wooded small tree up to 6 meter or more, but it is not hardy in nature. This plants was cultivated for leaf and flower colours and for oil production. Leaves are green or reddish in colour and about 30-60 cm in diameter. The leaves contain 5-12 deep lobes with coarsely toothed segments which are alternate and palmate. The stems are varying in pigmentation. The flowers are monoecious and about 30-60 cm. long[2].The fruit is a three-celled thorny capsule. The capsule of fruit covered with soft spins like processes and dehiscing in to three 2-valved cocci. The seeds are considerable differences in size and colour. They are oval, somewhat compressed, 8-18 mm long and 4-12 mm broad. The testa is very smooth, thin and brittle. Castor seeds have a warty appendage called the caruncle, which present usually at one end from which runs the raphe to terminate in a slightly raised chalaza at the opposite end of the seed[3]. This plant is common and quite wild in the jungles in India and it is cultivated throughout India, chiefly in the Madras, Bengal and Bombay presidencies. Two varieties of this plant are known A perennial bushy plant with large fruits and large red seeds which yields about 40 P.C of oil. A much smaller annual shrub with small grey (white) seeds having brown spots and yielding 37% of oil.

PHARMACOLOGY

Antioxidant activity

It is concluded that *R. communis* antioxidant activity by using lipid method and free radical scavenging effect on 2, 2 picrylhydrazyl radical (DPPH) and hydroxyl hydrogen peroxide. The high antioxidant activity of the seed of *communis* at low concentration shows that it could be very useful for the treatment of disease resulting from oxidative stress. The responsible chemical constituent of antioxidant activity are Methyl ricinoleate, Ricinoleic acid[4] octadecadienoic acid and methyl ester stem and leave extracts also produce antioxidant activity due to the presence of flavonoids in their extracts

Antinociceptive activity

The methanolic leaves extract of antinociceptive activity against formalin induced paw licking and The antinociceptive activity showed due to the presence preliminary Phytoconstituents like saponins, steroids and alkaloids -5-en-3-ol, stigmasterol, Y-sitosterol, fucosterol; essential oil using capillary like α-thujone (31.71%) and 1,8- (12.92%) and 30-Norlupan-3β-ol-20-one are bean[5]. seed extracts produced the per oxidation by ferric thiocyanate 2,2-diphenyl-1- radical generated from *R. communis* which produce 12- ester[6]. The *Ricinus communis* extracts[7,8]. *R. communis* possesses significant acetic acid induced writhing test, tail immersion methods in mice. Alkaloids[9].

Antiasthmatic activity

The ethanolic root extract of *R. communis* is effective in treatment of asthma because of its antiallergic and mast cell stabilizing potential effect. Saponins has mast cell stabilizing effect and the flavonoids possess smooth muscle relaxant and bronchodilator activity; the apigenin and luteolin like flavonoids were generally inhibit basophil histamine release and neutrophils beta glucuronidase release, and finally shows in-vivo antiallergic activity. The *R. communis* ethanolic extract decreases milk induced leucocytosis and eosinophilia and possess antiasthmatic activity due to presence of flavonoids or saponins[10].

Anti-fertility activity

The methanol extracts of *R. communis* seed possess positive preliminarily Phytochemical tests for both steroids and alkaloids. The pituitary gland releases gonadotrophins due to Sex hormones by both positive and negative feedback mechanism and also the pituitary gland block the release of luteinizing

hormone (LH) and the follicle-stimulating hormone (FSH) because of the effect of combined oestrogen and progesterone in the luteal phase of the menstrual cycle. Finally it helps the inhibition of maturation of the follicle in the ovary and prevents ovulation. The sex hormone being steroidal compound's (phytosterols) and the presence of steroids in methanol extract of *Ricinus communis* seed produces anti-fertility effects[11,12].

Antihistaminic Activity

The ethanol extract of R. communis root resulted anti histaminic activity at the dose 100, 125, and 150 mg/kg intraperitoneally by using clonidine induced catalepsy in mice[13].

***In vitro* immunemodulatory activity**

The plant and animal origin immunemodulatory agents generally increase the immune responsiveness of the human body against pathogens by activating the non-specific immune system. The phagocytosis is the engulfment of microorganism by leucocytes. In last the phagocytosis is the intracellular killing of microorganisms by the neutrophils. The presence of tannins in the leaves of *R. communis* significantly increased the phagocytic function of human neutrophils and resulted produces a possible immunemodulatory effect[14].

Hepatoprotective activity

Ricinus communis leaves ethanolic extract 250/500mg/kg body weight possesses hepatoprotective activity due to their inhibitory activities of an increase in the activities of serum transaminases and the level of liver lipid per oxidation, protein, glycogen and the activities of acid and alkaline phosphatase in liver induced by carbon tetrachloride (CCL_4). The *R. communis* ethanolic extract 250/500mg/kg body weight also treated the depletion of glutathione level and adenosine triphosphatase activity which was observed in the CCl4-induced rat liver. The presence of flavonoids in ethanol extract of *R. communis* produces beneficial effect the flavonoids have the membrane stabilizing and antiperoxidative effects. Hence the *R. communis* increase the regenerative and reparative capacity of the liver due to the presence of flavonoids and tannins. The anticholestatic and hepatoprotective activity was seen against paracetamol-induced hepatic damage due to the presence of N-demethyl ricinine isolated from the leaves of *Ricinus communis* Linn. The whole leaves of *Ricinus communis* showed the protective effect against liver necrosis as well as fatty changes induced by CCL4 while the glycoside and cold aqueous extract provide protection only against liver necrosis and fatty changes respectively[15-17].

Anti-inflammatory activity

Anti-inflammatory activities of the leaves and root extract were studied in Wistar albino rats in acute and chronic inflammatory models. The study indicated that the paw edema formation due to sub plantar administration of carragennan, characterizing the cellular events of acute inflammation. The 250 and 500 mg/kg dose of *R. communis* methanolic leaves extract possess protective effect in prevention of cellular events during edema formation and in all the stages of acute inflammation. The anti-inflammatory activity of *R. communis* methanolic extract was due to the presence of flavonoids because the flavonoids have the protective effect against carragennan-induced paw edema in rats[18-20].

Antimicrobial activity

The antimicrobial activities of *Ricinus communis* were good against dermatophytic and pathogenic bacterial strains *Streptococcus progenies, Staphylococcus aureus* as well as *Klebsiella pneumonia, Escherichia coli*. The result showed that the petroleum ether and acetone extracts possess good zone of inhibition whereas ethanolic extract having anti-bacterial activity only on higher concentration[21]. The different solvent extracts of roots of *Ricinus communis (*200 mg/ml) possess antimicrobial activity by using well diffusion method against pathogenic microorganisms such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Proteus vulgaris, Bacillus subtilis, Candida albicans* and *Aspergillus niger*. The hexane and methanol extracts showed maximum antimicrobial

activity where the aqueous extracts has no significant antimicrobial properties[22].

Antidiabetic activity

The ethanolic extract of roots of *Ricinus communis* (RCRE) was investigated along with its bioassay-guided purification. By Administration of the effective dose (500mg/kg b. w) of RCRE to the diabetic rats for 20 days possess favorable effects not only on fasting blood glucose, but also on total lipid profile and liver and kidney functions. Amongst all fractions the R-18 fraction suggests the significant antihyperglycemic activity. RCRE showed no significant difference in alkaline phosphatase, serum bilirubin, creatinine, serum glutamate oxaloacetate transaminases, serum glutamate pyruvate transaminases and total protein which was observed even after the administration of the extract at a dose of 10 g/kg b.wt. Thus *R. communis* is a potent phytomedicine for diabetes[23].

Wound healing activity

The *Ricinus communis* possess wound healing activity due to the active constituent of castor oil which produce antioxidant activity and inhibit lipid per oxidation. Those agents whose inhibits lipid per oxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibres, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis. The study of wound healing activity of castor oil was in terms of scar area, % closure of scar area and epithelization in excision wound model. Due to the astringent and antimicrobial property the tannins, flavonoids, triterpenoids and sesquiterpenes promotes the wound healing process, which are responsible for wound contraction and increased rate of epithelialisation. The study resulted that the Castor oil showed wound healing activity by reducing the scar area and also the epithelization time in excision wound model. The comparison study of two different concentrations (5%w/w and 10%w/w) of castor oil was resulted that the 10 % w/w Castor oil ointment possesses better wound-healing property[24].

Lipolytic activity

The ricin produces the lipolytic activity by using the various substrates: (i) one analogue of triacylglycerol, BAL-TC4; (ii) various chromogenic substrates such as *p*-NP esters of aliphatic short to medium chain acids, and (iii) monomolecular films of a pure natural diacylglycerol, DC10 in emulsion and in a Membrane-like model. The study concluded that ricin from *R. communis* act as a lipase and has the capability of hydrolyzing different lipid classes. Ricin also hydrolyses phospholipids which are the major components of cellular membranes. The lipolytic activities are maximal at pH 7.0 in the presence of 0.2 M galactose. The action of ricin on membrane phospholipids could occur through a phospholipase A1 activity which is very often a minor activity of lipases[25].

Molluscicidal, Insecticidal and Larvicidal activity

The leaf extract of ***R. communis*** possess molluscicidal activity against *Lymnaea acuminata* and the seed extracts showed better insecticidal and insectistatic activity than the leaf extracts against *S. frugiperda* due to the active ingredients like castor oil and ricinine[26, 27, 28]. The aqueous leaves extracts of *R. communis* possess suitable Larvicidal activity against *Anopheles arabiensis*, *Callosobruchus chinensis* and *Culex Quinquefasciatus* mosquitoes[29.]

Antiulcer activity

The castor oil of *R. communis* seed possess significant antiulcer properties at a dose of 500 mg/kg and 1000 mg/kg, but at the dose 1000 mg/kg was more potent against the ulceration caused by pylorus ligation, aspirin and ethanol in rats. The result showed that the antiulcer activity of *R. communis* is due to the cytoprotective action of the drug or strengthening of gastric mucosa and thus enhancing the mucosal defence[30,31].

R. communis or castor plant is a widely traditionally used and potent medicinal plant amongst all the thousands of medicinal plants. The pharmacological activities reported in the present review confirm that the therapeutic value of *R. communis* is much

more. It is an important source of compounds with theirs chemical structures as well as pharmacological properties. The presence of phytochemical constituents and pharmacological activities proved that the plant has a leading capacity for the development of new good efficacy drugs in future.

MATERIAL AND METHODS

Drugs and chemicals

The standard drug 5-FU purchased from Local Retail Pharmacy Shop and solvents and other chemicals used for the extraction and phytochemical screening were provided by Institutional Store and were of LR and AR grade.

Cell culture

The human prostate cancer cell line DU-145 cell line was provided by National Centre for Cell Science (NCCS), Pune and was 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.

Methodology for Soxhlet extraction

First the dried seeds are triturate to make fine powder and the powered material is placed into the thimble made of stout filter paper and the apparatus is fitted up. The flask containing suitable solvent like ethanol is heated on a water bath or on a heating mental. As the solvent boil, its vaporise rise through the side tube up into the water condenser. The condensed liquid drops on the solid in the thimble, dissolves the organic substances present in the powdered material and filters out into the space between the thimble and the glass cylinder. As the level of liquid here rises, the solution flows through the siphon back into the boiling flask. The solvent is once again vaporized, leaving behind the extracted substance in the flask. In this way a continuous stream of pure solvent drops on the solid material, extract the soluble substance and returns to the flask. At the end of the operation the solvent in the boiling flask is distilled off, leaving the organic substance behind[32]. Afterwards the ethanolic extract (EE-CS) transfer in a clean and dried beaker and is concentrated by placing on a water bath and then cool, keep it in a freeze. From this concentrated extract the preliminary phytochemical screening has to be carried out.

Phytochemical screening[33-36]

Preliminary phytochemical screening of EE-CS have shown the presence of diverse bioactive molecules such as: carbohydrates, proteins and aminoacids polyphenols, carotenoids, phytosterols and alkaloids which are confirmed by their specific qualitative cofirmatory chemical tests.

Principle[37,38]

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**.**

Procedure[39]

The monolayer cell culture was trypsinized and the cell count was adjusted to 0.5-1.0x105 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 and10 µg/ml, 20 µg/ml, 30 µg/ml and 40 µg/ml of different concentration of EE-CS were added to the cell in microtitre plate. The plates were incubated at 37^0c for 72 hrs in 5% CO2 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 40c 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. 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 EE-CS against DU-145 cell lines for various concentrations are shown in table 1. As the concentration increases there is an increase in the cell growth inhibition and it was found that EE-CS, with the highest 92.80% growth inhibition at 40 µg (IC_{50} = 2.5 µg/ml), with the 90.06% growth inhibition at 30 µg (IC_{50} = 2.9 µg/ml). The IC_{50} value of standard drug 5-FU was found to be 1.5 µg/ml with 96.54 % growth inhibition at concentration 50 µg/ml.

IC_{50} determination

IC_{50} is the acronym for "half maximal inhibitory concentration". IC_{50} value indicates the concentration needed to inhibit a biological or biochemical function by half (e.g. inhibition of enzymes, affinity to cell receptors). Amongst others, determination of IC_{50} is commonly calculated via linear interpolation: The activity of an enzyme is determined after exposure to a series of inhibitor concentrations. IC_{50} is calculated by the following formula:

IC_{50} = (50% - Low Inh%) / (High Inh% - Low Inh%) x (HighConc-Low Conc) + Low Conc.

Low Inh% / High Inh%: % inhibition directly below / above 50% inhibition

Low Conc / High Conc: Corresponding concentrations of test compound.

Table No.1: For percentage (%) of cell Growth Inhibition of EE-CS on DU-145 Cell lines by SRB Assay

S.No	Concentration of EE-CS	Absorbance of extracts	Inhibition of cell growth (%)
1	10 µg/ml	0.036	87.67
2	20 µg/ml	0.032	89.05
3	30 µg/ml	0.029	90.06
4	40 µg/ml	0.021	92.80
5	50 µg/ml (5-FU)	0.0101	96.54
6	Control	0.292	0

Figure No.1: Flowering plant

Figure No.2: Fruit

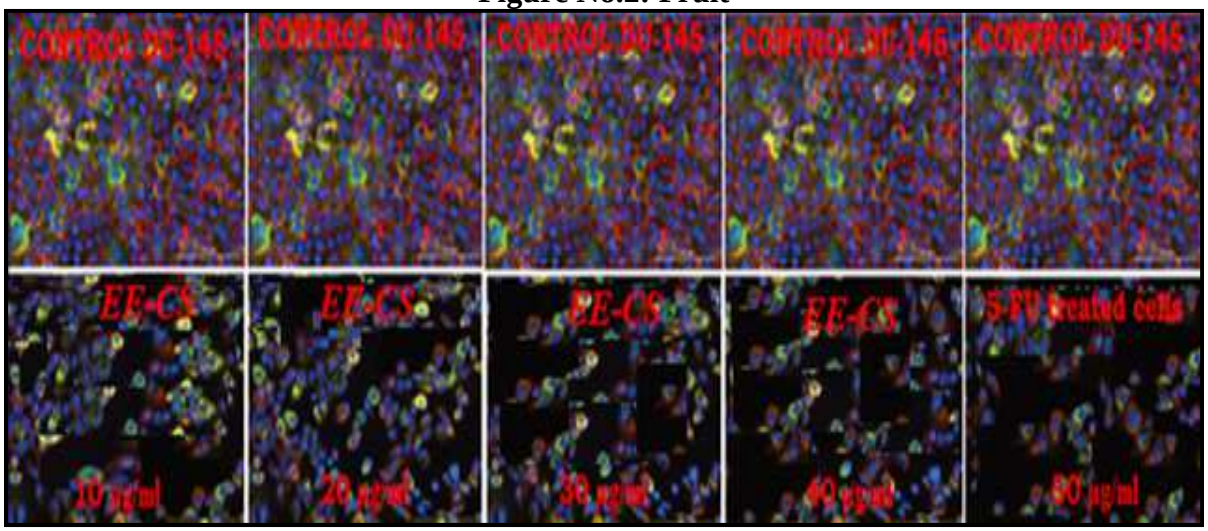

Figure No.3: Percentage (%) of cell growth inhibition by EE-CS on human prostate cancer DU-145 cell line

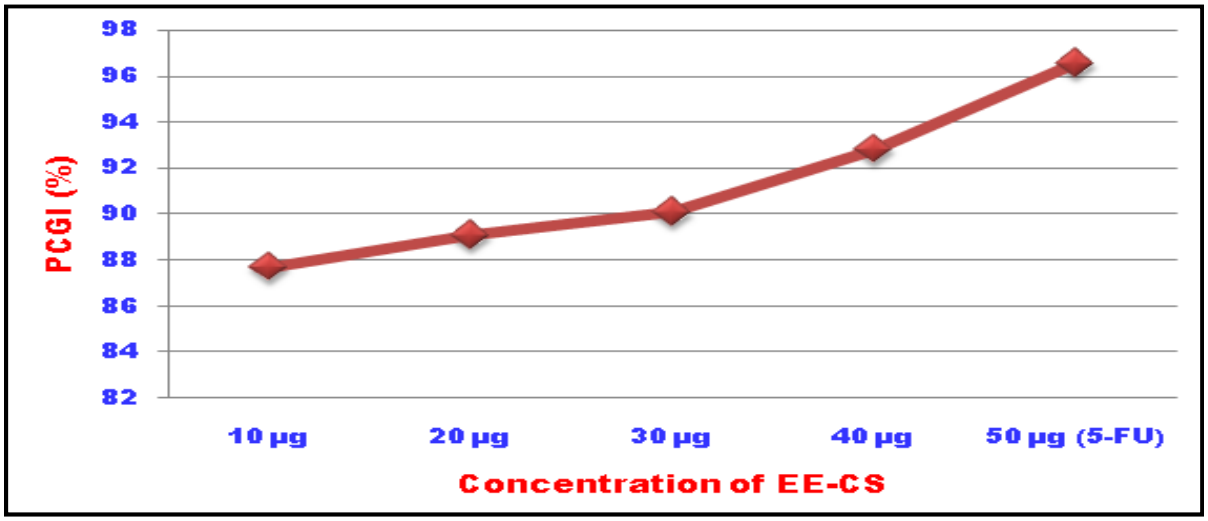

Figure No.4: Graphical representation of Percentage (%) of cell Growth Inhibition by EE-CS on DU-145 Cell line at different concentration

CONCLUSION

The results obtained from the present studies displayed that the Preliminary Phytochemical screening of EE-CS had shown the presence of carbohydrates, proteins and aminoacids polyphenols, carotenoids, phytosterols and alkaloids etc and the results obtained from the *in-vitro* studies performed by SRB assay against human prostate cancer cell line DU-145 displayed that the EE-CS possessed moderate to good anti cancer activity and exhibiting the potential capability to inhibit the cancer cell when compared with standard drug 5-FU.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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