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COSTUS SPECIOSUS AS ALTERNATIVE SOURCE OF MEDICINE FOR TISSUE REPAIR, NEOPLASMIC, METABOLIC DISORDERS, ALONG WITH ITS ANTI-HELMINTHIC AND ANTI-BACTERIAL ACTIVITY

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ABSTRACT

Costus speciosus is an important medicinal plant which can be used as alternative source of medicine for Diabetes, wound repair, against certain cancer cells and as antibacterial. The article reviews some of the techniques employed and the results obtained for the Anti-hyperglycaemic activity, Anti-neoplastic activity, Anti-helminthic, Anti-bacterial and wound healing activity. The techniques employed have been highlighted with emphasis on modern techniques and their advantage over common techniques.

Key words: *Costus Speciosus*, Anti-hyperglycaemic activity, Wound healing, Anti-neoplastic, anthelmintic.

INTRODUCTION

Medicinal plants have the capacity to produce secondary metabolites which in some cases protect the plant from pathogens but are also useful for treating various human ailments.

Costus speciosus have been traditionally used in the treatment of Diabetes, worm infestation. [1] Costunolide from roots of these plants are an important candidate for treating Type II Diabetes mellitus. Methanolic and aqueous extracts of plants shows promising results as Anti-helminthic. Diosgenin from the rhizomes have shown promising results as cytotoxic agents against HepG2 cell lines.

Antimicrobial activity of sesquiterpene lactone (costunolide and eremanthin) isolated from this plant is significant. Wound healing though not much studied, it is reported that juice extracted from the leaf of this plant is applied topically to heal wounds. Modern techniques employing genetically modified obese rats for testing the anti-hyperglycemic activity are useful. Anti-helminthic activity using earthworms along with modern techniques like docking can be performed. Cytotoxic studies employing HepG2 cells uses IC50 value comparison with paclitaxel. Docking studies for anticancer effects on other cell lines can also be employed. *Costus speciosus* is an important source of alternate medicine and various constituents from other parts of the plant need to be isolated and tested for pharmacological activity using modern techniques, also the economic significance to be considered for carrying such studies.

METHODS USED AND RESULTS OBTAINED

Anti-hyperglycemic

In one study ethanolic extracts of the plant was given orally (200 mg/kg) for 15 days to Alloxan treated rats using Glbenclamide as standard and biochemical analysis performed for TG (Triglycerides), HDL (High Density Lipoprotein), VLDL (Very Low Density Lipoprotein), LDL (Low Density Lipoprotein), protein, urea using commercial kits and blood glucose levels determined by glucose oxidase method. On the 14th day histological studies were done [2].

Another study, involves Streptozotocin induced diabetic rats. Costunolide an active constituent was isolated from the plant using TLC and then given to rats for 30 days to assess the effect on fasting blood glucose (normohypoglycaemic effect) and cholesterol levels at a dose of 20 mg/kg body weight. Glycosylated hemoglobin, plasma insulin, muscle glycogen, serum lipid and plasma were the variables studied. [3] Use of genetically modified obese rats are one step ahead in determining the

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antidiabetic activity since it employed the insulin tolerance test and glucose tolerance test, thus helping to determine the activity of the plant extracts in combination with insulin administration. [4] Student's t test, regular one-way analysis of variance helps in determining the significance of the result obtained. Multivariate analysis was performed to establish association between dependent variables and each study variable, Mauchly's test of sphericity with the use of epsilon correction or Pillai's trace estimator to assess if the models had the assumption of compound symmetry.

Alloxan induced model showed significant decrease in blood glucose, Glycosylated hemoglobin, blood urea, serum uric acid, serum creatinine, triglycerides, total cholesterol, phospholipids, Low density lipoprotein, very low density lipoprotein, and increase in liver glycogen, insulin and lactate dehydrogenase. The mechanism seems to be by enhancing the insulin secretion by the Islets of Langerhans, enhancing peripheral glucose utilization and increase serum protein levels.

STZ (Streptozotocin) induced diabetes costunolide produced effects similar to that of Alloxan induced model study. Costunolide seems to inhibit the nitric oxide synthase activity thus helped in correcting the secretory defects in diabetes.

The results obtained in the genetically modified rat model similarly indicated better glucose modulation when administered in compliment with insulin injection.
The use of genetically modified animal helped in determining the normohypoglycaemic activity.

Anti-helminthic

Costus speciosus rhizome juice have been given traditionally with sugar as anti-helminthic. Aqueous and Methanolic extract of aerial parts of the plant at the concentration of 25, 50, 100 mg/ml on adult Indian earthworm (*Pheretima posthuma*) showed significant paralysis which is dose dependent and increases with concentration when compared to Albendazole as standard. [5] The smooth muscle relaxant property is attributed to the paralysis effect. Other in vitro techniques are

1. Egg hatch test [6]
2. Larval development test
3. Adult motility assay.

In egg hatch test eggs were incubated at 27ºC after treatment with plant extract and the standard drug. 48 h later Lugol's solution was added to halt the hatching process, eggs and first stage larvae are counted. Larval development test involves measuring the inhibition after the first stage of larval development while adult motility test is the same as test performed on earthworms. *In vivo* test involves testing on sheep, homogeneously infected with nematode species and egg count reduction was calculated. Probit transformation is used for egg hatch and larval development test, SAS software can be used for adult motility assay. Molecular docking of the active constituent can be done to determine the antihelmintic activity, the ligand molecule can be designed using ChemDraw Ultra 6.0 and 3D coordinates using PRODRG server. [7]

Anti-neoplasmic activity

Diosgenin isolated from the rhizomes of *Costus speciosus* was used against cancer cells Hep G2 (human epidermoid cells-G2, hepatocellular carcinoma), MCF-7 (Michigan Cancer Foundation-7, adenocarcinoma), HCT-116 (Homo sapiens colon colorectal colon carcinoma) and cultured till confluence was reached to 75% similarly immune cell were cultured using Lymphoblastic leukemia 1301 cells and raw murine macrophage RAW 264.7 to produce stable cell lines and confluency achieved was 75%. The MTT (3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide) cell viability assay was used for measuring cytotoxicity. The number of viable cells is directly proportional to the level of soluble formazan dark blue color. Cytotoxicity was measured in relation to relative viability. Fluorescence analysis of Apoptosis and Necrosis in living cell to analyse the mode of death using EB (Ethidium Bromide) dye which is taken by cells whose structural integrity is lost. Live cells have abnormal green nucleus, early apoptotic cells have bright green to yellow color, and late apoptotic cells have fragmented orange colored chromatin.

Indirect immunoassay ELISA (Enzyme Linked Immunosorbent Assay) technique (by adding antibodies for DR-4 (Death Receptor-4) or capase-3 to cell lysate coated wells and using polyclonal goat anti-rabbit peroxidase conjugate and followed by substrate buffer the level of DR-4 was expressed as optical density values and compared with the activity of paclitaxel) was used for caspase-3 and death receptor-4 measurement in cell lysates. Proliferation index in lymphoblastic leukemia cells and raw murine macrophage was done using MTT assay and compared with standard (Echinacea purpurea roots) immunostimulant.

Statistical analysis can be done using IBM SPSS software. Cytotoxicity was found against all three cell lines used, highest in MCF-7 cell line (IC50 11.03 µg/ml), moderate for HL-60 cell line (IC50 22.98 µg/ml) and least for HepG2 cell line (IC50 32.62 µg/ml).

Cell cycle at 30% IC50 value showed predominant growth arrest at S- and G2/M PHASE.

Significant increase in the apoptotic and necrotic cell population was seen majority been apoptotic. Proliferation of lymphocytes was achieved at a concentration of 250 µg/ml. Sample showed significant and almost similar activity to that of paclitaxel in DR-4 induction but lower for caspase-3. [8]

Anti-bacterial

Sesquiterpene lactones isolated from *Costus speciosus* exhibits antibacterial activity. Costunolide and eremanthin were isolated from hexane extract by TLC (Thin Layer Chromatography) and identified by X-ray crystallography or GC-MS (Gas Chromatography-Mass Spectroscopic) analysis. Hexane, chloroform, Ethyl acetate, Methanol and water extracts were screened by disc diffusion assay method against *S. aureus, B. subtilis, and S. epidermidis.* Minimum inhibitory concentration was determined as the lowest concentration of the compound inhibiting the visual growth of bacteria.

Hexane extract inhibits the growth of *S. aureus* (15mm, 2.5 mg/disc), *S. epidermidis* (15mm, 5mg/disc), and *B. subtilis* (15mm, 2.5 mg/disc). The hexane extracts exhibited better activity compared to extracts in other solvents. [9-11]

Wound healing and cellular repair

Wound healing activities have not been much studied in *C. Speciosus*. Leaf of this plant is mixed with the leaves of *Cynodon dactylon*, *Glycyrrhiza glabra*, *Canna indica* and stem bark of *Punica granatum*. The juice extracted from this mixture is applied topically on affected places to heal wounds. Some of the methodologies employed in wound healing are given below.

A high-throughput cell migration assay using, scratch wound healing assay of tissue culture cell monolayer modified by use of 384 well formats. Mechanically scratching the cell array scratch is made on all the wells and the healing process is studied. Imaging of the healing wounds with an automated fluorescence microscope allows us to distinguish perturbations that affect cell migration, morphology, and division. Readout requires ~1 hr. per plate but is high in information content i.e. high content. [12]

The adaptation of a wound healing assay to a 384 well format facilitates the study of aspects of cell migration, tissue reorganization, cell division, and other processes that underlie wound healing. This assay allows greater than 10,000 perturbations to be screened per day with a quantitative, high-content readout, and can also be used to characterize small numbers of perturbations in detail.

In vivo wound models are done on rats and guinea pigs. They are basically three types [13-16].

1. Excision wound model.
2. Deep space wound model.
3. Burn wound model.
4. Incision wound model.

Excision wound model was used for the study of the rate of contraction of wound and epithelization. Incision wound model differs from excision wound model in that the wounds are left open in later while the wounds were tightly sutured in the former. Deep space wound model studies the physical and mechanical changes in the granuloma tissue by inflicting subcutaneous dead space wounds. Burn wound models uses brass bricks to produce rectangular wounds. The progressive changes in the wound were monitored by camera on predetermined days.

The following parameters are basically measured

a. Measurement of wound contraction.
b. Determination of period of epithelization.
c. Tensile strength.
d. Measurement of wound index.
e. Estimation of collagen (hydroxy proline), hexosamine, hexuronic acid.
f. Granuloma studies.
g. Estimation of protein and DNA content.

In vitro tests are relatively inexpensive and can be used to screen a wide variety of samples simultaneously but are incapable of replicating all the factors responsible for wound healing.

The various in vitro assay are

1. Chick chorioallantoic membrane (CAM) assay.
2. Fibroblast Assay.
3. Keratinocytes Assay
4. Scratch assay.
5. Collagen lattice formation.
6. Electrical healing assay.
7. Collagen assay.
8. MPO assay.
9. Hyaluronidase inhibition assay.
10. Collagenase and elastase inhibition assay.

In the CAM model the angiogenic activity of extracts are studied on 9-day old fertilized chick eggs. The photographic images of the CAM model are analyzed for quantitative morphometric analysis of the density of blood capillaries in terms of the number of red pixel per unit areas using Image J software and Angio Quant software.

Fibroblast bioassay uses Human dermal fibroblast cells from post auricular surgery and cultured. The neutral red assay is used to analyse the effect of extracts on the growth of fibroblasts.

Keratinocytes assay uses residual skin samples isolated during surgery. After treatment and thorough washing epidermal sheets are peeled from the dermis and minced and dispersed in Trypsin solution proliferation activity of fibroblast are stopped prior to culturing. Scratches are made on the cultured cells and the healing process is noted as distortions of the main part of the scratch.

Scratch assay is a simple method which involves making of scratch on the monolayer and recording the closing of gap to study the migration rate by comparison of the images.

Study of the agent which slows down contraction which is the final stage of wound healing and the cause of scar formation can be studied in collagen lattice formation. Electrical healing assay uses electric cell-substrate impedance sensing result in severe wound healing process.

CONCLUSION

Costus speciosus is an important medicinal plant whose whole part can be used for isolation and characterization of active constituents; it has shown promising results as anti-hyperglycemic, anti-cancer, anthelmintic, and anti-bacterial. Studies on wound healing activities need to be done in detail. Stem and floral parts need to screened for active compound isolation moreover callus and tissue cultures need to be analyzed for pharmacological activities.

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