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RESEARCH ARTICLE

CYTOTOXICITY AND ANGIOGENIC ACTIVITY OF *CANSJERA RHEEDII* J.GMELIN

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ABSTRACT

Despite clinical advances in anticancer therapy, there is still a need for novel anticancer metabolites, with higher efficacy and lesser side effects. The present study evaluated the anticancer, angiogenic activity, phytochemical compounds, and antimicrobial activity of the plant *Cansjera rheedii* J.Gmelin. *Cansjerarheedhii (opiliaceae*) is a climbing shrub, Whole plant of *Cansjerarheedhii* was used by the tribes of Auroville village near Puducherry for various liver disorders. For checking the cytotoxicity, trypan blue assay result shows the presence of active compounds in the ethanolic plant extract. This is confirmed by MTT assay which gives an IC50 value of 150µg/ml on MCF-7 and 150µg/ml on HT-29 cell lines. The phytochemical study of ethanolic extract of *Cansjera rheedii* showed the presence of alkaloids, carbohydrates, flavonoids, phenols, saponins, amino acids, terpanoids and tannin. Ethanolic plant extract shows more active in antimicrobial and angiogenicactivity.Future studies are necessary for chemical characterization of the active principles and more extensive biological evaluation.

Key words: Phytochemicals, Cytotoxicity, Angiogenecity, Antibacterial, Trypan blue assay, MTT assay.

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INTRODUCTION

Cancer is a major scourge of society and an important public health problem. It also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity (Ebana *et al.,* 1991, Pamplona and Roger Williams, 1996, 1999, Manna and Abalak 2000). According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Joshi *et al.,* 2010). *Cansjerarheedhii* (Opiliaceae) is aclimbing shrub, sometimes armed, commonly known as Kalimankeerai in Tamil is generally found in India through Malaya to Hong Kong and North Australia (Gamble 1981; Mathew 1991). Whole plant of *Cansjerarheedhii* was used by the tribes of Auroville village near Puducherry for various liver disorders. In earlier studies, the ethanol extract of aerial parts of *C.rheedii* has been reported to have hepatoprotective, cytotoxic, anthelmintic, anti-inflammatory and membrane stabilizing property, antipyretic, antinociceptive and diuretic activities (Mounnissamy *et al.,* 2009). Hence the present work has been designed to investigate the phytochemical compounds, cytotoxic activity, antibacterial activity and angiogenic activity present in water and water+ethanol extract of *Cansjera rheedii* due to its enormous therapeutic uses.

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MATERIALS AND METHODS

Plant material: The bark of *Cansjera rheedii* was collected from Kolli hills of Namakkal, Tamil Nadu district during July-August 2017 and the plant species was authenticated and the herbarium was deposited. The plant material were washed thoroughly in tap water, shade dried and powdered and used for extraction.

Preparation of the extract: The plant sample was extracted directly with water as well as with absolute ethanol: water at the ratio of 1: 1 by maceration for 72 hrs. The extracts were concentrated by rotary vacuum evaporator (Yamato RE300, Japan) and then dried. The percentage yield was expressed in terms of air dried weight of plant material. The evaporated extracts thus obtained were dissolved in the respective solvents at the concentration of 1mg/ml and used for further studies.

Antimicrobial Activity

Microbial strain: The following bacterial and fungal strains were obtained from MTCC and NCIM and screened according to Bergey's Manual of Bacterioloy (Holt *et al.,* 1994). *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumonia, Salmonella typhi*

Agar well diffusion method: Agar well diffusion assay was performed to investigate antibacterial efficacy of SBCR

against bacteria was inoculated into a sterile plate with 20ml Muller Hinton agar and the plate was shaken for even spread and proper mixing of the organisms and agar. It was then allowed to solidify. 4wells of approximately 6mm in diameter were made on the surface of the agar plate using a sterile borer. The plates were then turned upside down and the wells were labeled with a marker and a volume (25-100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then agar plates were incubated at 37°c for 24hrs and zone of inhibition was measured.

Anticancer Activity

Cell line: The human colorectal adenocarcinoma cell lines (HT 29), MCF-7 (human breast, adenocarcinoma) was obtained from Amala Cancer Centre,Kerala and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0c, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passage weekly, and the culture medium was changed twice a week.

MTT assay: MTT (3-(4,5 Dimethylthiazol)-2,5- Diphenyl Tetrazolium Bromide) assay, is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MIT and form a dark blue formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Addition of a detergent results in the liberation of the crystals, which are solubilised. The colour can be spectrophotometrically measured. The level of the coloured formazan products is directly proportional to the number of surviving cells. (Mosmann *et a1*., 1983). The cytotoxicity of the extracts was tested against MCF 7 (human breast adenocarcinoma cell line) and HT 29(human colorectal adenocarcinoma). Cells harvested in the log phase of growth were counted and seeded (5 x 103 cells/well in 100 µl) in 96 well titre plates. PBS was added to the outer wells (200µl/well). After 24 hours of incubation at 37°C in 5% CO2 to allow cell attachment (in the case of adherent cells), cultures were treated with varying concentrations (18.5-300µg/m1) of working solution of CR1 in medium. Each treatment was carried out in triplicates. Untreated cells served as negative control. 5Fu (10µg/m1) was used as a positive control for viabilities studies. The plates were incubated for 48 and /or 72 hours. On completion of incubation, media were removed from wells without disturbing the cells. To each well 100µ1 of 1mg/ml solution of MTT were added and plates were incubated for 2 hours in dark at 37°C in a CO2 incubator. 100 µl of lysis buffer was added to each well and the plates were further incubated for 4 hours in dark in a CO2 incubator. After the incubation period, absorbance was read at 570 nm using a multiwall plate reader and % of cytotoxicity was calculated.

Trypan Blue Exclusion Test: Trypan blue dye exclusion assay was performed as a screening test for cytotoxic potential of the various extracts prepared. Different concentrations of *Cansjera rheedii* (1000, 500, 250, 125, 62.5 µg/ml) was added to the cells and then made up to 1 ml with PBS. Cells were incubated for 3 hours at 37 C. After incubation, the cell death was evaluated using trypan blue exclusion method. To the cell suspension, 3 drops of trypan blue (0.5 % in PBS) was added and the cells were loaded immediately on to a haemocytometer. The number of dead cells was counted and the percentage of dead cells was calculated. Viable cells exclude the dye while non-viable cells take up the dye and appear bluein colour. The percentage growth inhibition was calculated and CTC value is generated from the dose-response curves for each cell line.

% Of Cytotoxicity = (No. of blue cells/Total no. of cells) X 100

Angiogenesis

Embryonated chicken eggs were collected from Suguna Chickens, Udumelpet, Tamilnadu. The egg shell of eight day eggs was gently cut open to expose the aeravasculosa. Paper discs soaked in various concentrations (5µl, 25µl,40µl and 50µl) and individual discs were placed directly on the blood vessel and incubated in room temperature for two days. The change in width of vessels was monitored for each concentration.

RESULTS AND DISCUSSION

Antimicrobial Activity

Agar Well Diffusion Method: The bark extract of this plant was tested for antimicrobial activity by agar well diffusion assay against six important microbial strains. The antimicrobial activity was studied by measuring the inhibition zone around the well. The highest zone of inhibition at 1:1 concentration was reported. Among the concentrations of bark extract of *Cansjera rheedii* as interpreted form the zone size, All organisms showed susceptibility towards both extracts. The zone of inhibition was largest (16mm) at 1:1 concentration. The ethanolic extract showed higher activities against *Sreptococcuspyogenes.* When the sample prepared with DMSO, the largest zone of inhibition was 20mm agaist *Streptococcus pyogenes, Staphylococcus aureus, Salmonella typhi, klebsiella*sp., and *Pseudomonas* sp.

Anticancer activity: Short term cytotoxicity was assessed by incubating $1x10^6$ DAL cells in 1mL phosphate buffered saline (PBS) (Appendix) containing various concentrations of *C. rheedii*extracts (1000,500,250,125,62.5 µg/mL) at 37 °C for 3 h. In the preliminary screening with trypan blue dye exclusion assay ethanol extract (ST1) was found very active. Thus it was further analysed using MTT assay which gave an IC50 value of 150µg/ml on MCF-7 and on HT-29 Cell lines. The death of treated cells also analysed by acridine orange ethidium bromide dual staining. So many treated cells apperared orange after staining, indicating dead cells. Some orange cells showed condensed chromatin membrane blebbing which indicated apoptosis.

Trypan blue assay

Cytotoxic property of *Cansjera rheedii:* 50% ethanolic extract on DAL cells by trypan blue exclusion method.

Table (a). In vitro cytotoxic studies of CR S1 WE on DAL cells

Test Drug	Test concentration (µg/ml)	Viable cells (number)	% growth inhibition
CR S1 WE	1000	18	80
	500	59	57
	250	96	34
	125	109	23
	62.5	69	9
Control	-	-	6

Table (b). In vitro cytotoxic studies of CR S2 W on DAL cells

Test Drug	Test concentration (µg/ml)	Viable cells (number)	% growth inhibition
CR S2 W	1000	16	79
	500	51	54
	250	82	29
	125	107	25
	62.5	61	5
Control	-	-	6

MTT assay

Table (a). MTT Assay on MCF-7 Cell line

Test concentration (µg/ml)	Growth inhibition (%)	IC50 (µg/ml)	R2
18.75	17.04		
37.5	45.23		
75	58.64		
150	77.86	52.07	0.97
300	94.50		

Table (b). MTT assay on HT-29 cell lines

Test concentration (µg/ml)	Growth inhibition (%)	IC50 (µg/ml)	R2
18.75	15.08		
37.5	40.90		
75	53.29		
150	70.17	45.67	0.91
300	86.98		

Angiogenesis: Angiogenic activity of ethanolic extract of *Cansjera rheedii* stem bark was determined, strongly elicited on angiogenic response which is visible with microscope as a spoke-wheel-like pattern of blood vessels. Application of 50µl of ethanolic extract of *Cansjera rheedii* stem bark exhibit great antiangiogenesis comparing to other concentration (5µl, 25µl, 40µl). The ethanolic extract of *Cansjera rheedii* on chick embryonic angiogenesis was increased dose dependently.

Conclusion

The result of the present investigation reports the presence of phytochemical compounds, antibacterial activity, cytotoxic activity and angiogenic activity of stem bark extract (ethanolic 1:1) of *Cansjera rheedii*. More research needs to be done to reveal the inhibitory effect of this plant. Future studies can be performed for the development of new drugs for various diseases.

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