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CYTOGENETIC ACTIVITY STUDIES ON SOME MANGROVES OF KRISHNA–GODAVARI ESTUARY

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ABSTRACT

In the present study the bark of Ceriops decandra, collected from godavari estuary, Ceriops tagal and Xylocapus molluccensis collected from Krishna estuary of hexane, methylene chloride and methanol extracts were evaluated for Genotoxicity and cytotoxicity effects. The results indicated that the extracts from the all the three mangrove plant species have cyto-toxicity and it is substantiated from mitotic index and cell proliferation kinetics values. Further, the extracts indicated that they are geno-toxic and it is substantiated by an increase in sister chromosome exchange frequency.

KEYWORDS: **Mangroves, *Ceriops decandra,Ceriops tagal*, *Xylocarpus moluccensis*, Genotoxicity, cytoxicity.**

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INTRODUCTION

Cytogenetic and related changes in human cancer constituent part of constantly developing and enlarging genetic alterations associated with cancer development and biology[1]. The most convenient and readily available tissue for cytogenetic study is peripheral blood. The optimization of the procedure for obtaining chromosome spreads from peripheral blood culture represents a significant milestone in the history of cytogenetics[2]. The growth inhibitory effect of the extracts from sixteen species of mangrove plants in Hainan on HeLa Cells. The AcOEt Extracts of *X.granatum* showed growth inhibitory activity on Hela Cells[3].*Xylogranatin* A-D, Novel Tetranortriterpenoids with an unusual 9, 10-*seco* Scaffold were evaluated for the Cytotoxic activities against two tumor cell lines, P-388 marine leukemia and A-549 human lung Carcinoma[4]. Five limonoids granaxylocarpins A-E, Xyloccensin U were studied the cytotoxic effect against the tumor cell line P-388 and A-549 by using MTT and SRB Methods[5]. Xylomexicanin A and Xylomexicanin B were tested[11] for antiproliferative activity against human breast carcinoma Cells (KT), While Xylomexicanin B did not show inhibitory effect on eleven human tumor cell lines[6].three new protolimonoids protoxylocarpins F-H along with 11 known compounds from seed kernels of *Xylocarpus granatum* were tested for cytotoxic activity against five human tumor cells[7]. The MeOH Extracts of the barks and pneumatophores of *Xylocarpus moluccensis* were assessed for their effects on the central nervous system[8]. Comparative antimicrobial activity studies on hexane, chloroform and methanol extracts of *Ceriops decandra* mangrove medicinal plant was carried out, using Disk Diffusion Assay, the MeOH extracts of C.decandra showed prominent antimicrobial activities, while chloroform and hexane extracts show very less or no antimicrobial activity[9]. Antihyperglycaemic activity of crude extract of *Ceriops tagal* leaves studied (investigated) in normoglycaemic and streptozotocin- induced diabetic rats[10]. The ethanolic extracts of a mangrove plant *Ceriops tagal* and its sequential fractions were studied for their effect on H^3-2-deoxyglucose uptake by L_6 rat muscle cells[11].

MATERIALS AND METHODS

Plant material Collection

The fruits of Ceriops tagal and *Xylocarpus moluccensis* were collected from Kothapalem Krishna estuary (15^0 53^1 latitude and 82^0 longitude), Andhra Pradesh on April 2008. The stem bark of Ceriops decandra was collected from Corangi Mangrove forest near Bhiravapalem of Godavari estuary (16^058^1 N latitude and 82^0 15^1 E longitude), Andhra Pradesh in 2008.

Identification

Xylocarpus moluccensis, the mangrove plant was identified by Dr. A. J. Soloman Raju, Dept of environmental sciences, Andhra University. Voucher specimen (code Ech1/101) have been deposited at the Engineering chemistry, AU College of Engineering, Andhra University Visakhapatnam.

Extraction

The fruits (3kg) of Xylocarpus molluccensis, the fruits (3kg) of Ceriops tagal and the stem bark of Ceriops decandra (3kg) were shade-dried, cut into small pieces and powdered. The powder was exhaustively extracted successfully with n-hexane, dichloromethane and methyl alcohol. Removed the solvent by vacuum distillation and obtained three extracts from each plant respectively thus totally nine extracts.

Materials

RPMI 1640 (GIBCO), PHA- phytohemagglutinin (GIBCO), Colchicine (Sigma), 0.75M KCl (Qualigens) Methanol (Qualigens), Glacial acetic acid (Qualigens), BrdU (Sigma), Hoechst 3325 (Sigma), Giemsa's stain, Sorenson's buffer, 2XSSC, Sterile slides, Torsons 15mL tubes and glass droppers.

Methods

The most convenient and readily available tissue for cytogenetic study is peripheral blood. The optimization of the procedure for obtaining chromosome spreads from peripheral blood culture by Moorhead et al. and Nowell represents a significant milestone in the history of cytogenetic.

Sample collection

A sample of blood (0.5 ml) was collected from the left hand vein of the communicating author by venipuncture and it was placed into a heparinised (green top, sodium heparin) tube. The sample thus collected was immediately for conducting the experiment.

Experimental Procedure

Peripheral Blood Culture Initiation

A sample of blood (0.5ml) collected from the left hand vein of author of the thesis was cultured in medium RPMI 1640 and a mitogen phytohemagglutinin (PHA) 100µL was added. And 1µg of plant extract and 1µg of compound added to the blood cultures. [12]BrdU (200µg) was added after 24th hour of the cultures. Peripheral blood cultures were incubator for 72hrs at 37°C.

Harvesting of the blood cultures

[13]colchicine was added at the end of the incubation. The cultures were incubated for 40 minutes after addition of colchicine and at 1200 RPM centrifuged for 10 minutes. hypotonic solution (0.75M KCl) 6mL added to the pellet and incubated for 10 minutes and at 1200 RPM centrifuged for 10 minutes. 5 ml of fixative solution (3:1 ratio of methanol and acetic acid) was added to the pellet and centrifuged. Fixative washes were given for 4-5 times. The fine pellet was dropped onto chilled sterile and slides.

Sister Chromatid Exchange assay

After the third day of the preparation of the slides, were used for Sister Chromatid Exchange assay. [13]After the third day of the preparation of the slides, were used for Sister Chromatid Exchange assay. For this assay kept a drop of Hoechst 3325 solution onto the slide and mounted with cover slip for 25 minutes. Then, the slides were kept for drying in dark chamber after washed with water. The slides were exposed to UV for 40 minutes after placed in Petri dish which contains 2XSSC, for 25 minutes. The slides were washed with water and dried for a few minutes. The slides were incubated in 2XSSC at 56°C for 2hrs and stained them with 2% Giemsa's stain with Sorenson's buffer.

Giemsa Staining

Solid staining is sometimes called conventional staining which produces nonbanded preparations. This method has been largely obsolete since the introduction of banding methods in the late 1960s and early 1970s. However, for selected applications, it may still be utilized. The scoring of chromosome and chromatid breaks and gaps, for example, can be facilitated by solid staining. Satellites, secondary constrictions, dicentrics, ring chromosomes, double minutes, and fragile sites can be better visualized by solid staining. Chromosome morphometry can also be better performed with nonbanded chromosomes. The stains employed are usually one of the Romanovsky-type dyes such as Giemsa, Leishman's, or Wright's stains. Stain the slides with Giemsa's stain and screen the slides under light microscope. Calculate the Mitotic index (MI) and scored the chromosomal abnormalities using light microscope.

Scoring of the all parameters including MI, CPK and SCE, is done using Upright Light Microscope as follows

Cell-cycle specific patterns were determined by MI, M2, and M3 metaphases. These were defined by the number of cell cycles completed. MI metaphases substituted BrdU unifilarly in both chromatids without differential staining. Chromosomes of M2 metaphases contained a bifilar substitution of BrdU in one chromatid, which, consequently, was lightly stained, thus displaying the typical sister chromatid differentiation pattern. Bifilar incorporation of BrdU in the third cell cycle yielded light staining of both chromatids. Such metaphases were classified as M3 metaphases. The cell

proliferation kinetic index is calculated using the following formula

$$.CPK = \frac{M_1+2M_2+3M_3}{100}$$

SCE

The SCE frequency including exchanges in the centromere was counted in M2 metaphases.

Mitotic Index

[14]Mitotic index is a measure for the proliferation status of a cell population. It is defined as the ratio between the number of cells in mitosis and the total number of cells. Cell population growth occurs as the cells pass through interphase and mitosis to complete the cell cycle.[15]Cell population growth occurs as the cells pass through interphase and mitosis to complete the cell cycle. Many cells lose the tendency to divide as they mature or divide only rarely. Other cells are capable of cell division rapidly.
Mitotic index was calculated by using the formula:

$$\text{Mitotic index} = \frac{\text{No. of dividing cells}}{\text{Total no. of cells}} \times 100$$

Statistical Analysis

Student t-test and Anova tests were used for data analysis purpose. Data were expressed as mean (± sdv.). The pair wise differences were computed by Student's *t*-test.

RESULTS

Table -01 shows MI and CPK values of cultures exposed to plant extracts of *Xylocarpus moluceensis* Fruits, Hexane Extract (Ex-1), Methyl alcohol Extract (Ex-2) and Methylene Chloride Extract (Ex-3). *Ceriops tagal* fruits of Hexane Extract (Ex-4), Methyl alcohol Extract (Ex-5), and Methylene Chloride Extract (Ex-6). *Ceriops decandra* stem bark of methylene Chloride Extract (Ex-7), Methyl alcohol Extract (Ex-8) and Hexane extract (Ex-9). The mean value of MI of control of 5.44 whereas plant extracts 1µg, 2µg and 3µg per culture has MI of 3.43, 3.06 and 2.80 respectively. So, when compared to control values plant extracts increased induced significant mito-depression. CPK value of control group is 0.48. 1µg, 2µg and 3µg of plant extract exposed cultures shows CPK values 0.45, 0.39, and 0.31 respectively.

Table 1
Results from Mitotic Index and CPK Studies on Plant Extracts

S. No	Sample	1µg/culture		2µg/culture		3µg/culture	
		MI	CPK	MI	CPK	MI	CPK
1.	Ex-1	3.13	0.42	2.87	0.35	2.62	0.26
2.	Ex –2	3.22	0.46	2.72	0.38	2.58	0.28
3.	Ex -3	3.75	0.45	3.4	0.46	2.91	0.36
4.	Ex–4	3.83	0.42	3.34	0.34	3.1	0.26
5.	Ex -5	3.43	0.48	2.92	0.48	2.83	0.38
6.	Ex -6	3.52	0.46	3.4	0.36	3.15	0.39
7.	Ex -7	3.45	0.47	3.15	0.46	2.9	0.37
8.	Ex -8	3.37	0.44	2.95	0.36	2.68	0.26
9.	Ex -9	3.25	0.47	2.8	0.37	2.44	0.27
	Mean ± STD Dev	**3.43±0.2311**	**0.45±0.02**	**3.06±0.26**	**0.39±0.05**	**2.80±0.23**	**0.31±0.05**
10	**Control**	**5.44**	**0.48**	**5.44**	**0.48**	**5.44**	**0.48**

Control vs Extracts - (1µg, 2 µg and 3µg /culture)
MI = Mitotic Index
CPK = Cell Proliferation Kinetics

Table 2
Results from SCEs Studies on plant Extracts

	1µg/culture	2µg/culture	3µg/culture
Extract – 1	10.2	13.56	17.68
Extract – 2	9.08	12.35	15.21
Extract – 3	11.21	15.93	19.52
Extract – 4	10.92	16.35	17.68
Extract – 5	11.44	13.55	16.53
Extract – 6	11.62	15.52	18.88
Extract – 7	12.11	12.32	18.32
Extract – 8	10.61	14.57	17.9
Extract – 9	9.99	16.12	16.99
Mean ± STD Dev	**10.79 ±0.93**	**14.47 +/- 1.59**	**17.63 ±1.28**

P<.01 Control SCE's 5.86.
SCE = Sister Chromatid Exchanges

Table-02 shows the results of Plant extract induced a significant number of Sister Chromatid Exchanges (SCE's) in doses 1µg, 2µg and 3µg for culture when compared to control culture. The mean Sister Chromatid Exchange frequency in control is 5.86, whereas 1µg, 2µg and 3µg of Plant extracts induced 10.79**,** 14.4, 17.63 SCE's per metaphase on an average. These results are significantly different from control values.

The metaphase of Extracts of plants

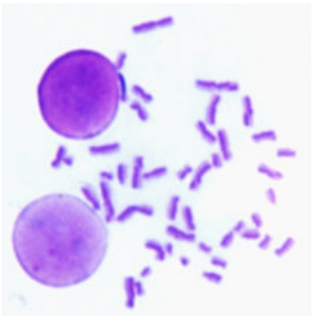

Figure: 01 The metaphase from the control culture showing few SCEs

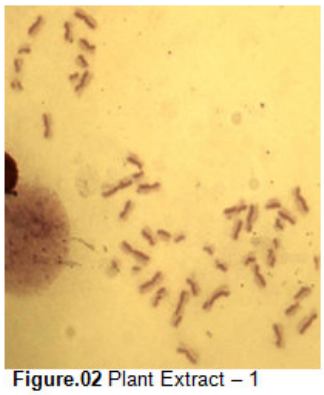

Figure.02 Plant Extract – 1

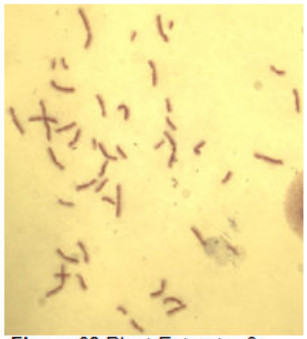

Figure.03 Plant Extract – 2

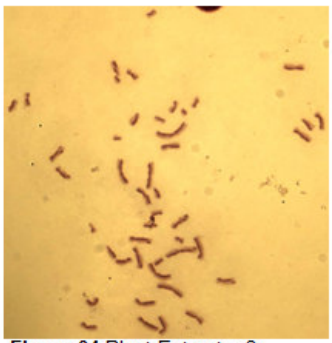

Figure.04 Plant Extract – 3

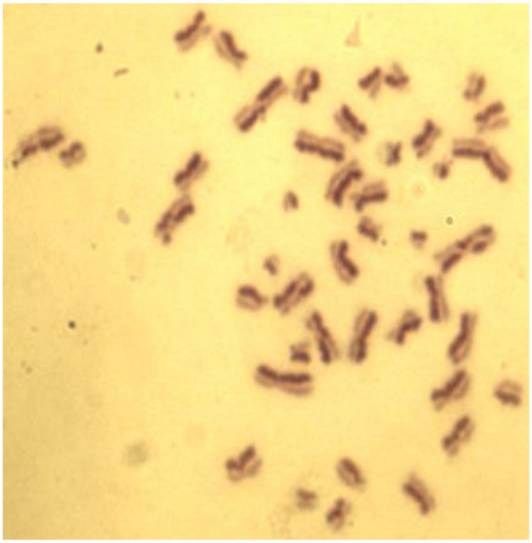

Figure.V-05 Plant Extract – 4

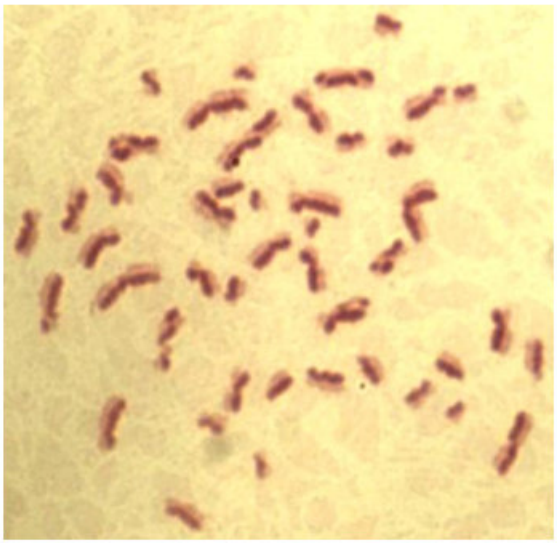

Figure.06 Plant Extract – 5

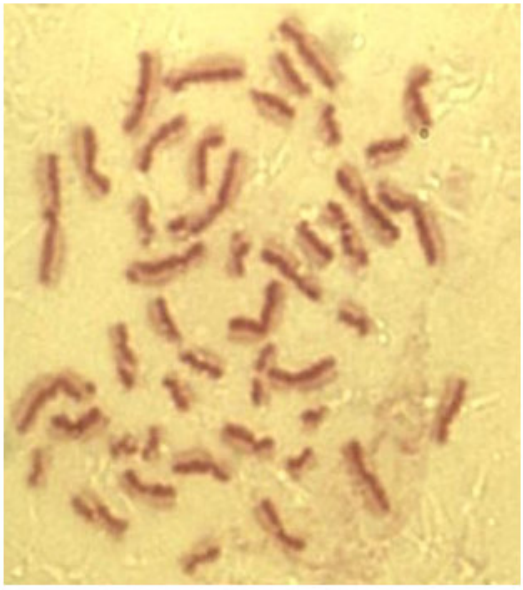

Figure.07 Plant Extract – 6

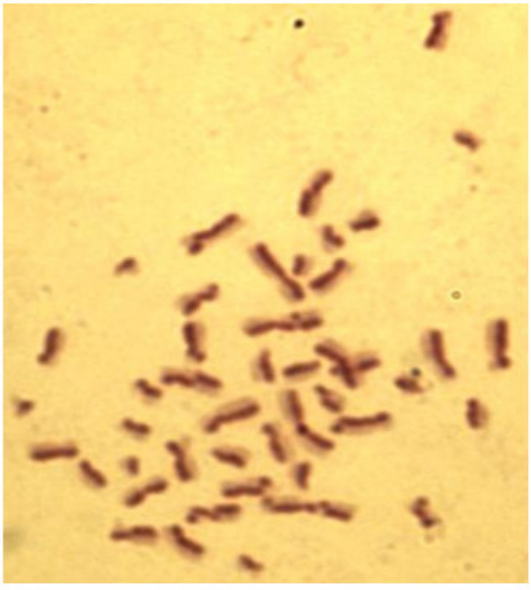

Figure.8 Plant Extract – 7

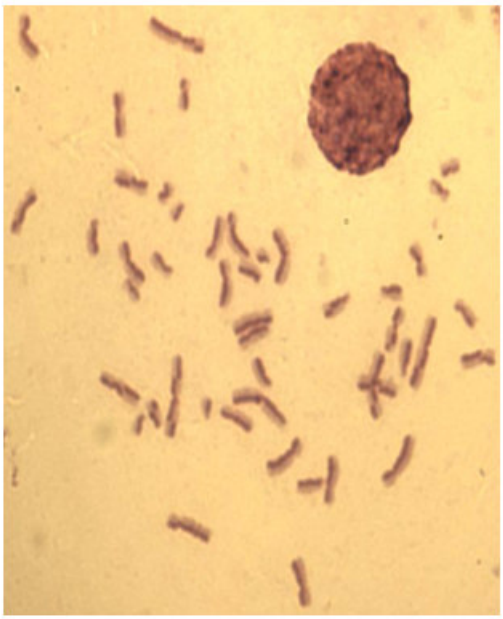

Figure 09 Plant Extract – 8

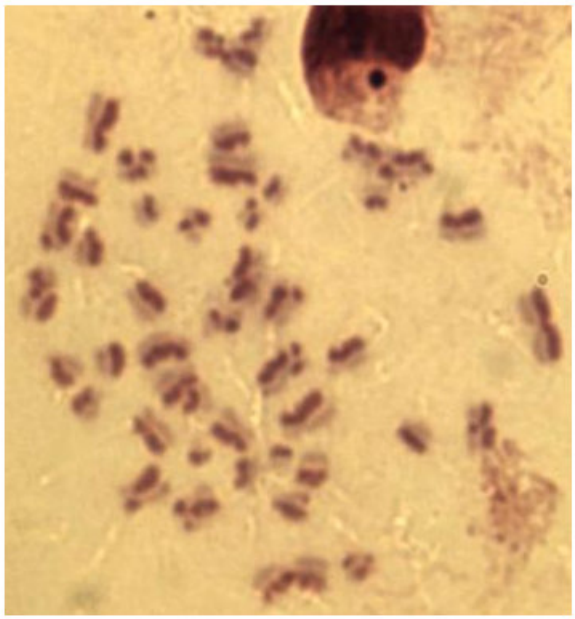

Figure.-10 Plant Extract – 9

DISCUSSION

The results indicated that the extracts from the all the three mangrove plant species have cyto-toxicity and it is substantiated from mitotic index and cell proliferation kinetics values. Further, both the extracts and compounds indicated that they are geno-toxic and it is substantiated by an increase in sister chromosome exchange frequency. The present study clearly indicated that from the stem bark of *Ceriops decandra* and fruits of *Ceriops tagal* and *Xylocarpus moluccensis* are mutagenic with clear cut dose response. The findings of the present study would form the baseline information for intensive and extensive studies in this line in order to evaluate their efficacy in drug formulations for treating human diseases, especially for cancer drug formulations. However, there is a limitation for using these plant species since they grow in restricted and highly specialized environment in estuarine areas. *C. tagal* is a rare species while its sister species *C. decandra* is a common species which occurs in almost all mangrove forests of India and elsewhere. *X. moluccensis* is quite uncommon and grows in restricted salinity zonation. Of the three plant species, there is a huge potential to consider any cancer drug formulations from *C. decandra* since it is a common and easily obtained from any mangrove forest

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