Research Article

Prospective Anticancer Efficacy of Bioactive Compounds from *Chicoreus ramosus* (Linnaeus, 1758)

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Submission Date: 05-06-2023; Revision Date: 18-08-2023; Accepted Date: 14-09-2023.

ABSTRACT

Aim: For over three decades, marine creatures have been a rich source of novel chemical compounds that have accelerated the development of marine natural product chemistry. Among the marine organisms, molluscs are one of the most successful forms of beast life and they've conquered nearly every niche and live in all the abysses. In the present study, marine gastropod *Chicoreus ramosus* have been selected with a view to screen the bioactive compounds responsible for biological activities. **Materials and Methods:** The cytotoxic effects of the experimental organism were performed using an MTT assay on the HeLa cell line. The chance of cell viability was set up to be dropped by adding attention to the samples. **Results:** The result of GC-MS results showed 10 compounds responsible for antioxidant and anticancer activities The results of the present study revealed that *Chicoreus ramosus* showed potent cytotoxic activities against HeLa cell lines with IC_{50} values of218.60 µg/mL. **Conclusion:** Methanolic extract of *Chicoreus ramosus* yielded a pool of bioactive compounds which are responsible for anticancer activity. Thus, it may be helpful to the pharmacological technologist for the extraction of anticancer drugs in the near future.

Keywords: Anticancer activity, *Chicoreus ramosus*, GC-MS analysis, HeLa cell line.

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INTRODUCTION

Cancer is a group of conditions involving abnormal cell growth that frequently develops the ability to spread to other parts of the body. As an example, elisidepsin, a novel marine-derived cyclic peptide belonging to the Kahalalide family of compounds currently in phase II development with preliminary evidence of antitumor activity, is one of the anticancer compounds currently being tested in clinical trials.[1]

The effectiveness of natural anticancer drugs has been evaluated using a wide range of *in vivo* and *in vitro* techniques. MTT assay and Sulforhodamine B assay are the most widely used *in vitro* techniques for determining anticancer activity.[2] A number of cancer types may develop as a result of interactions between unrestrained revolutionaries and DNA, resulting in mutations that may harm the cell cycle and maybe produce malice.[3]

Under typical physiological circumstances, DNA is continuously subject to endogenous and external sources of damage, necessitating the coordinated effort of numerous DNA form mechanisms to maintain genomic integrity.[4] A number of genomic abnormalities, including point mutations, chromosomal translocations, and gain-of-function mutations, can be impacted by a failure to repair DNA damage accurately and promptly.[5] So, the present study has been carried out with a view to analyze the bioactive compounds through GC-MS analysis and to evaluate the anticancer efficacy of the marine gastropod *Chicoreus ramosus*.

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DOI: 10.5530/ajbls.2023.12.53

MATERIALS AND METHODS

Collection of experimental animals

In the present study, the gastropod *Chicoreus ramosus* was procured from the Gulf of Mannar coastal region. The neogastropod *C. ramosus* was collected from the landed by-catch of fishing trawlers operated for crabs and prawns along the Thoothukudi coastal region. Fresh sea water was used to clean and wash the recently acquired samples at the laboratory in order to get rid of any contaminants. The shells were cracked, the tissues were taken out, and they were then dried in a hot air oven for 48 hr at 56°C.

GC–MS analysis

The following conditions, such as Column elite - 5MS fused silica capillary column (30 x 0.25mm ID × 0.25m df, composed of 5% Diphenyl 95% Diphenyl Poly Siloxane), were used in GC-MS analysis on a GC Clarus 500 Perkin Elmer System using an AOC 20i Autosampler and gas chromatography interfaced to a mass spectrophotometer (GC-MS) instrument: As a carrier gas, helium (99.999%) was utilized at a constant flow rate of 1 mL/min, a volume of injection of 3 L (10:1), and a temperature of 250°C. The oven temperature was set to rise from 110°C (isothermal for 2 min.) to 200°C, then to 280°C at a rate of 10°C/min. At 70eV, a scan interval of 0.5 sec, and fragments ranging in size from 45 to 450 Da, mass spectra were recorded.

Identification of compounds

The National Institute of Standard Technology (NIST Ver. 21), WILEY 8 and FAME databases, which include more than 62,000 patterns, were used to interpret the mass spectrum. The spectrum of the known components contained in NIST, WILEY, and FAME, the MS library, and anticipated from Duke's Ethno Botanical Database were compared to the spectrum of the unknown components discovered in the body tissues of *Chicoreus ramosus*.

Anticancer activity (MTT assay)

Human cervical cancer cells were used in the MTT 3- (4, 5- dimethyl thiazol - 2- yl) - 2, 5 - diphenyl tetrazolium bromide assay to investigate the cytotoxic effects of the methanol extract of the experimental animal *Chicoreus ramosus*. The HeLa cell line was given by the National Centre for Cell Sciences (NCCS), which is based in Pune, India. Stock cells were grown in DMEM with 10% inactivated Foetal Bovine Serum (FBS), 100 g/mL of penicillin, 100 g/mL of streptomycin, and 5 g/mL of amphotericin B at 37°C until confluent. TPVG solution (0.2 trypsin, 0.02 EDTA, and 0.05 glucose in PBS) was used to separate the cells. All studies were conducted in 96 microtitre plates and the stock cultures were grown in 25cm^2 culture flasks (Tarsons India Pvt. Ltd., Kolkata, India). Using DMEM with 10% FBS, the monolayer cell culture was trypsinizied and the cell density was increased to 1.0×10^5 cells/mL. A total of 0.1 L of the diluted cell suspension approximately (10,000 cells) was added to each well of the 96-well microtitre plate. The supernatant was removed after 24 hr when a partial monolayer had formed and washed once before various test drug concentrations were applied to the partial monolayer trypsinization of the monolayer cell culture and DMEM containing 10% FBS was used to increase the cell density to 1.0×10^5 cells/mL. The diluted cell suspension (approximately 10,000 cells) was put into each well of the 96-well microtitre plate in a quantity of 0.1 mL. After 24 hr, when a partial monolayer had developed, the supernatant was removed, the plate was washed once, and various test drug concentrations were applied to the partial monolayer to produce final concentrations of 50, 100, 150, and 200 g/mL. The plates were then incubated for 3 days at 37°C in a 5% CO_2 atmosphere, during which time a microscopic examination was conducted and observations were recorded every 24 hr. After 72 hr, the drug solutions in the wells were discarded and 50µL of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 hr at 37°C in a 5% CO_2 atmosphere. To dissolve the formazan that had developed, the supernatant was withdrawn, 100 L of propanol was added, and the plates were gently agitated. At a wavelength of 570 nm, the absorbance was calculated using a microplate reader. The control cells were those that weren't treated. The following formula was used to express the samples' impact on the proliferation of HeLa cells as a percentage of cell death.

$$\% \text{ of cell death} = \frac{\text{Control OD} - \text{Treated OD}}{\text{Control OD}} \times 100$$

DNA fragmentation assay on HeLa Cell line

The Tyagi *et al.,* (2014) method was used to conduct the DNA fragmentation experiment.[6] HeLa cell lines were cultured in a 5% CO_2 incubator for 24 hr together with a crude extract of *C. ramosus*. Trypsin Phosphate Versene Glucose Reagent (TPVG) was added after incubation, and the mixture was then centrifuged at 15,000 rpm for 10 min. Proteinase K was used to wash the pellet, and it was then incubated at 55°C for 3 hr. After that, phenol, chloroform, and isoamyl alcohol were added in the proportions of 25:24:1 v/v, rapidly vortexed, and

incubated on ice for 5 min. The aqueous layer was then transferred to a fresh Eppendorf tube, and the phenol, chloroform, and isoamyl alcohol extraction was carried out once more after centrifugation at 10,000 rpm for 10 min. The aqueous layer was mixed with 2.5 mL of 100% cold ethanol and 50 mL of 3M sodium acetate before being overnight kept at -20°C. It was then centrifuged for 5 min. at 4°C at a 15,000 rpm speed. The bullet took 5–10 min to air dry. The dried powder was then reconstituted in 100 L of buffer (10 mM Tris/1 mM EDTA) before being run through a 1% agarose gel. Ethidium bromide (1 g/mL) was used to stain the gel. The clear bands were seen, captured and photographed.

RESULTS

GC-MS Analysis

The sample was subjected to GC-MS analysis. GC-MS analysis from the animal *Chicoreus ramosus* revealed 10 compounds that could be identified as Hydrazinecarboxylic acid, Phenol, 4-(1-methyl ethyl)-acetate, Propanedinitrile, 2,2-Dimethyl propionic acid, Cyclopropane,1-(4-methoxyphenyl), Chloroacetic acid, 3-tridecyl ester, 3-(4-methoxyphenyl)propionic acid, trans-4-methoxychalcone, 4-Hexylanisole and 4-cholesten-3-one semicarbazone.

Chemical component structures present in the crude methanolic extract of *C. ramosus* were depicted in Table 1 respectively, which could be responsible for antioxidant and anticancer activities.

Anticancer Activity

The methanolic extract of *C. ramosus* showed anticancer activity in the range of 15.72% to 46.02% at different concentrations of 50-200 µg/mL (Table 2). The percentage of cell death was found to be 15.72% at 50 µg/mL, 23.84% at 100 µg/mL, 36.68% at 150 µg/mL and 42.02% at 200 µg/mL (Figures 1 and 2).

Table 1: Activity of components identified in the column fractions of *Chicoreus ramosus* by GC-MS.

Sl. No.	RT	Name of compound	Molecular formula	Mw g/mol	Area	Compound nature	Activity	Structure
1	6.843	Hydrazinecarboxylic acid	$CH_4N_2O_2$	76.055	72495	Monocarboxylic acid	Anticancer, Immunomodulators, Antioxidant	
2	7.855	Phenol,4-(1-methylethyl)-acetate	$C_9H_{12}O$	136.194	336996	Monoterpenoid phenol	Antibacterial, Antioxidants, Anticancer	
3	8.384	Propanedinitrile	$C_3H_2N_2$	66.06	214189	Alkanenitrile	Anti-infectives, Antibacterial	
4	8.639	2,2-Dimethyl propionic acid	$C_5H_{10}O_2$	178.231	96790	Carboxylic acid ester	Antiviral, Anticancer	
5	12.336	Cyclopropane1-(4-methoxyphenyl)	$C_{11}H_{11}NO$	173.215	96790	Carboxylic acid	No activity	
6	13.377	Chloroacetic acid, 3-tridecyl ester	$CH_2ClCOOH$	94.494	242833	Carboxylic acid	Antioxidant Anti-inflammatory	
7	14.738	3(4-methoxyphenyl) propionic acid	$C_{10}H_{12}O_3$	180.203	271258	Carboxylic acid	Antibacterial, Immunostimulants, Specific for leukemia	
8	15.031	Trans-4-methoxychalcone	$C_{16}H_{14}O_2$	238.286	432822	Aromatic ketone	No activity	
9	16.497	4-Hexylanisole	$C_{13}H_{20}O$	192.302	130748	Hydroxy ketone	Wound healing, Analgesics	
10	17.490	4-Cholesten-3-one semicarbazone	$C_{27}H_{44}O$	384.648	843504	Cholesterol oxidase	Antibacterial, Anti-inflammatory	

Table 2: Anticancer activity of *Chicoreus ramosus*.

Sl. No.	Concentration (µg/mL)	Absorbance of control	Absorbance of sample	% of cell death	IC_{50} value
1	50	1.145	0.965	15.72	218.60
2	100	1.145	0.872	23.84	
3	150	1.145	0725	36.68	
4	200	1.145	0.618	46.02	

µg-micro grams; mL-millilitre; %-Percentage; IC_{50}-Inhibitory concentration.

Figure 1: MTT assay.

MTT-3-(4,5 Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; DMSO-Dimethyl Sulfoxide; DMEM-Dulbecco's Modified Eagle Medium; µg-micro grams; mL-millilitre.

Sample	Blank – DMSO	Blank – DMEM medium	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL
Control	Blank – DMSO	Blank – DMEM medium	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL

The highest percentage of cell death was observed at maximum concentration and the lowest percentage of cell death was recorded at minimum concentration. It was observed that anticancer activity was found to be increased with increasing concentration. The IC_{50} value was found to be 218.60 µg/mL.

DNA Fragmentation on HeLa Cell Line

The result of the DNA fragmentation on the HeLa cancer cell line treated with experimental organism *C. ramosus* used agarose gel electrophoresis is shown in Figure 3. In this experiment, DNA was extracted from HeLa cell lines after 24 hr incubation with various concentrations (10 and 20 µg/mL) of *C. ramosus*. The result showed a clear ladder pattern formed at all concentrations, while no DNA fragmentation was observed in the control cells.

DISCUSSION

Marine secondary metabolites and marine natural products are gaining popularity. Researchers from a variety of fields, including marine biology, biochemistry,

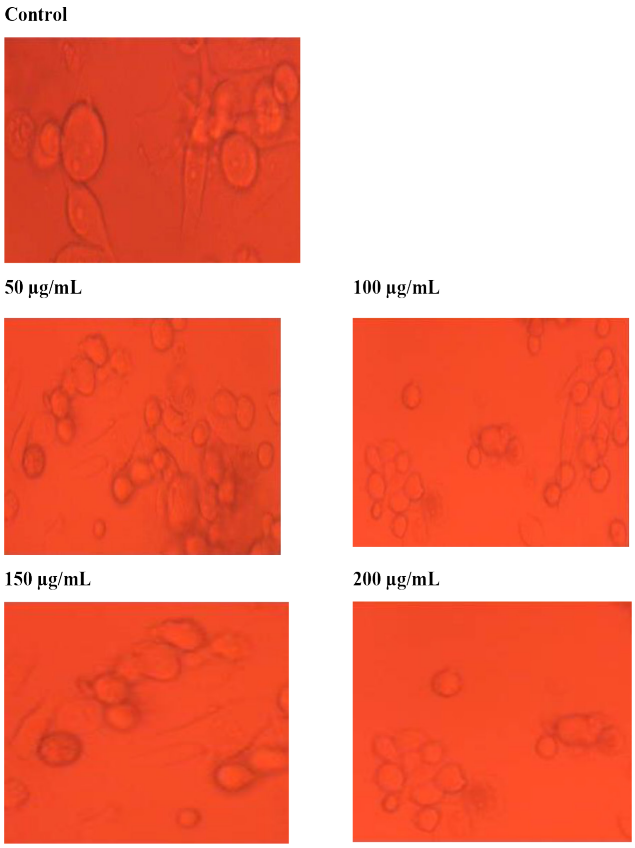

Figure 2: Anticancer Activity – HeLa cell line.

pharmacology, and biotechnology, are interested in this area of research. various chemicals with potential for use in medicine have been discovered through investigations on marine natural products over the past few decades, which has increased interest in this environment as a source of new drugs among various research groups.[7]

The presence of various bioactive composites justifies the use of whole animals for various ailments by traditional practitioners. Chemical drugs may lead to adverse effects and recent researchers have focused on pharmacologically active compounds from natural sources. GC-MS is used to identify the ingredients of unpredictable matter, long-chain and fanned-chain hydrocarbons, alcohols, acids and esters.

Manilal *et al.,* (2010)[8] studied the bioactive compounds present in the simple ascidian *Microcosmus exasperates*

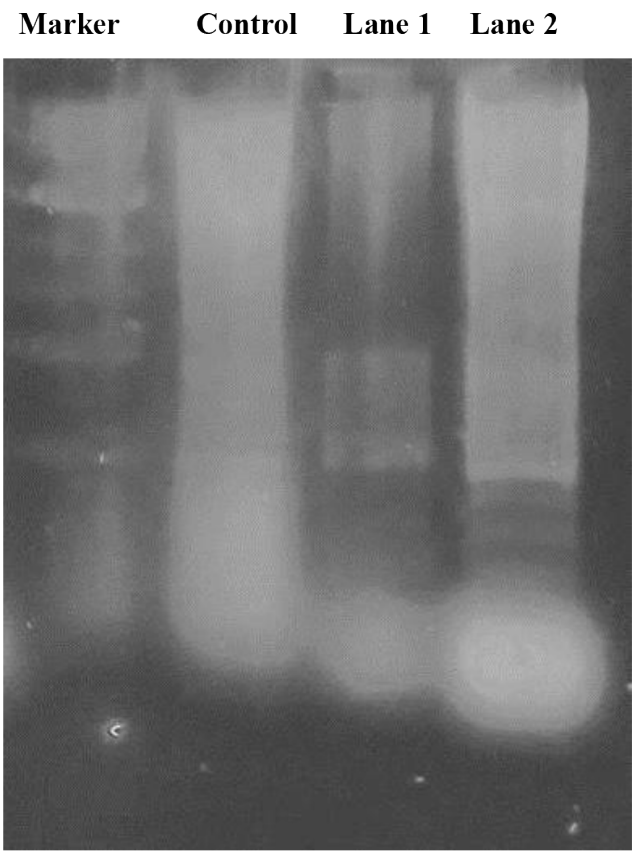

Figure 3: DNA Fragmentation Assay.
Marker-1500 bp; Lane 1-10 µg/mL; Lane 2-20 µg/mL;
bp-base pair; µg-micro gram; mL-milli litre.

and revealed the presence of 20 chemical compounds having various biological activities. Nuzhat Afsar *et al.,* (2012)[9] reported the GC-MS analysis of fatty acids of prosobranch gastropod species *Thais carinifera* from Pakistan Coast. Divya Dharan (2018)[10] identified the bioactive compounds from the solvent extracts of *Aplidium multiplicatum* using GC-MS analysis. GC-MS chromatogram of the methanolic extract of *Aplidium multiplicatum* showed 21 peaks which indicates the presence of 21 chemical compounds with various biological activities like anti-microbial, anti-inflammatory, pesticide, chemoprevention, diuretic and antioxidant.

In the present study, 10 chemical constituents have been identified from methanol extract of the whole animal of *C. ramosus* by gas chromatogram mass spectrometry (GC-MS) analysis (Table 1). The 10 compounds reported viz., Hydrazinecarboxylic acid, Phenol, 4-(1-methyl ethyl)-acetate, Propanedinitrile, 2,2-Dimethyl propionic acid, Cyclopropane,1-(4-methoxyphenyl), Chloroacetic acid, 3-tridecyl ester, 3-(4-methoxyphenyl)propionic acid, trans-4-methoxychalcone, 4-Hexylanisole and 4-cholesten-3-one semicarbazone. Similar findings were reported by Gayathri *et al.,* (2017)[11] in freshwater snail *Pila virens*, Jemma Hermalin Jesy Diaz *et al.,* (2015)[12] in Cephalopods and Subavathy *et al.,* (2016)[13] in *Cypraea arabica*. The present study corroborates well with the above findings.

Cancer is still a dreaded disease, which accounts for 9% of the deaths throughout the world. It is one of the 10 leading causes of death today, in India. One important case in finding new anti-cancer compounds is that much attention is paid to side effects caused by chemotherapeutic drugs. With regard to the considerable cytotoxic goods reported from marine brutes in recent decades, the search for new composites with considerable anti-cancer goods has increased (Majid Honari *et al.,* 2017).[14]

In the present study cytotoxic effect of methanol extract of *C. ramosus*, MTT assay was performed using HeLa cell line. In the present study, the percentage of cell death was found to be 15.72% at 50 µg/mL, 23.84% at 100 µg/mL, 36.68% at 150 µg/mL and 42.02% at 200 µg/mL with IC_{50} value of 218.60 µg/mL. It was observed that the percentage of cell death was found to be increased with dose-dependent concentration. Subavathy *et al.,* (2021)[15] showed the cytotoxic effect of three gastropods *Turbo brunneus*, *Cypraea annulus* and *Babylonia spirata* on MCF-7 Cell Line. The current study agrees well with the above findings.

Kjelland *et al*., (2016)[16] studied DNA fragmentation in *Mytilus edulis* and Jacky Bhagat *et al*., (2016)[17] showed DNA damage and oxidative stress in *Morula granulata*. In the present study, DNA was extracted from HeLa cell lines at two different concentrations of 10 µg/mL and 20 µg/mL. The result showed a clear ladder pattern formed at all the concentrations. The present study corroborates well with the above findings.

So, the present study indicates that the marine gastropod *C. ramosus* could be effectively used as an alternative source of anticancer compounds with subsequent health benefits. The drugs from marine animals are more effective than any other forms. It is suggested that more research should be done on these animals which have more therapeutic uses for humans.

CONCLUSION

The results of the current investigation indicate that the marine gastropod *Chicoreus ramosus* inhibits the proliferation of the HeLa cell line. The study indicated that, in contrast to the control, DNA isolated from the HeLa cell line at two distinct concentrations had a ladder pattern. Future research on the purification and chemical elucidation of the active ingredient found in

the extract will pave the way for either the base or the creation of a brand-new medicine.

ACKNOWLEDGEMENT

The authors are grateful to the Principal of St. Mary's College (Autonomous), Thoothukudi for providing the necessary facilities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MTT: 3- (4, 5- dimethyl thiazol - 2- yl) - 2, 5 - diphenyl tetrazolium bromide; **HeLa:** Human Cervical Cancer Cells; **NCCS:** National Centre for Cell Sciences, Pune; **DMEM:** Dulbecco's Modified Eagle Medium; **FBS:** Fetal Bovine Serum; **TPVG:** Trypsin Phosphate VerseneGlucose; **EDTA:** Ethylenediamine tetraacetic acid; **PBS:** Phosphate Buffered Saline; **CO_2:** Carbon-di-oxide; **hr:** Hour; **C:** Celcius; **µL:** Micro litre; **µg:** Micro gram; **IC_{50}:** Inhibitory concentration.

SUMMARY

The present study describes the anticancer activity of *Chicoreus ramosus*. The marine gastropod exhibits strong anticancer action, opening the door for a novel medication in the near future.

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Cite this article: Priya PK, P. Subavathy, Dominikal AMA. Prospective nticancer Efficacy of Bioactive Compounds from *Chicoreus ramosus* (Linnaeus, 1758), *Antheraea assamensis* Helfer. Asian J Biol Life Sci. 2023;12(2):402-7.