In-Vitro Determination of the Anti-Mitotic Activity of *Gracilaria salicornia* (marine red algae) on *Tripneustes gratilla* (sea urchin) Embryos using Vincristine Sulfate as Control

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Background of the Study

Cancer

- 3rd leading cause of mortality in the Philippines

- life-threatening disease

- rapid cell division

Background of the Study

- The impact of cancer is a burden for the patient and for the society as a whole.

- The cost of anti-cancer drugs have been increasing.

- Discovery of affordable but similarly efficient alternative treatment would be beneficial.

Marine macroalgae

- Found to be an exceptional source of active pharmacological compounds which have the following activities:
  - Antineoplastic
  - Antimicrobial
  - Antiviral
  - Cytotoxic

Gracilaria salicornia

- abundant
- closely related to *Portierra hornemanii*
- no documented anti-mitotic activity

Tripneustes gratilla (sea urchin)

- large eggs
- easily available
- Cost-effective
- shares 7000 genes with human DNA

Related Studies

- In 1992, Lyngbye revealed that *Portiera hornemanii* contained "halomon" which exhibits cytotoxicity against tumor cell lines.
- In 2006, Luardo demonstrated the anti-mitotic activity of *Portiera hornemanii* on onion root tip cells.

Related Studies

- In a study in 2009, Notarte and associates screened different species of algae to test the strength of their antimitotic activity.

Rationale

- *Gracilaria salicornia* may be a possible treatment for cancer.

- Used as springboard for future studies.

Objective of the Study

To determine whether *Gracilaria salicornia* possess anti-mitotic activity on *T. gratilla* embryos using Vincristine sulfate as control.

Specific objectives

1.To compare the mean cell stages observed 165 minutes post administration of the test treatments:

- 100% filtered sea water (**negative control**)
- 20 mcg/ml Vincristine sulfate (**positive control)**
- 4% *Gracilaria salicornia* (**experimental group**)

Specific objectives

2. To compare the proportion of average cell stages that shows inhibition of mitosis defining sensitivity of the sea urchin embryos to the test substances.

METHODOLOGY

Study Design

- In-vitro controlled experimental study design

Study Setting

- University of San Carlos Marine Station in Maribago, Lapu-Lapu
- Microbiology department of Cebu Institute of Medicine

Study Population

Inclusion Criteria

•Only fresh *T. gratilla* sea urchins with an average diameter of 8-10cm

Exclusion Criteria

•Other species of marine algae and sea urchin.

Sampling Procedure

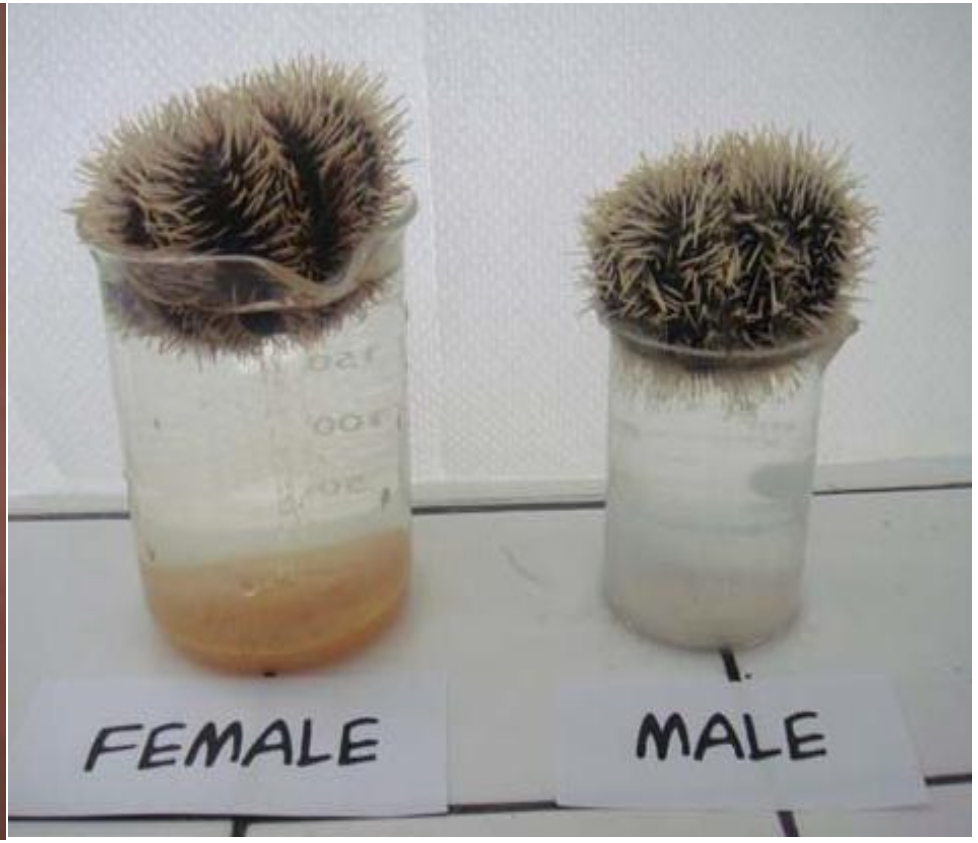
Sample Size:

A sample size of 750 was calculated.

Preparation of Marine Macroalgae (*Gracilaria salicornia*)

Preparation of Sea Urchin (*Tripneustes gratilla*)

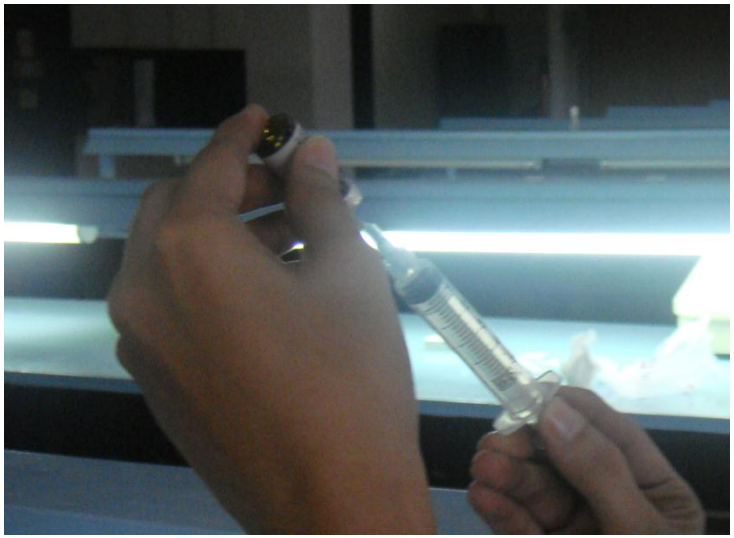
Spawning of the Sea Urchins (*Tripneustes gratilla*)



Experimental treatments

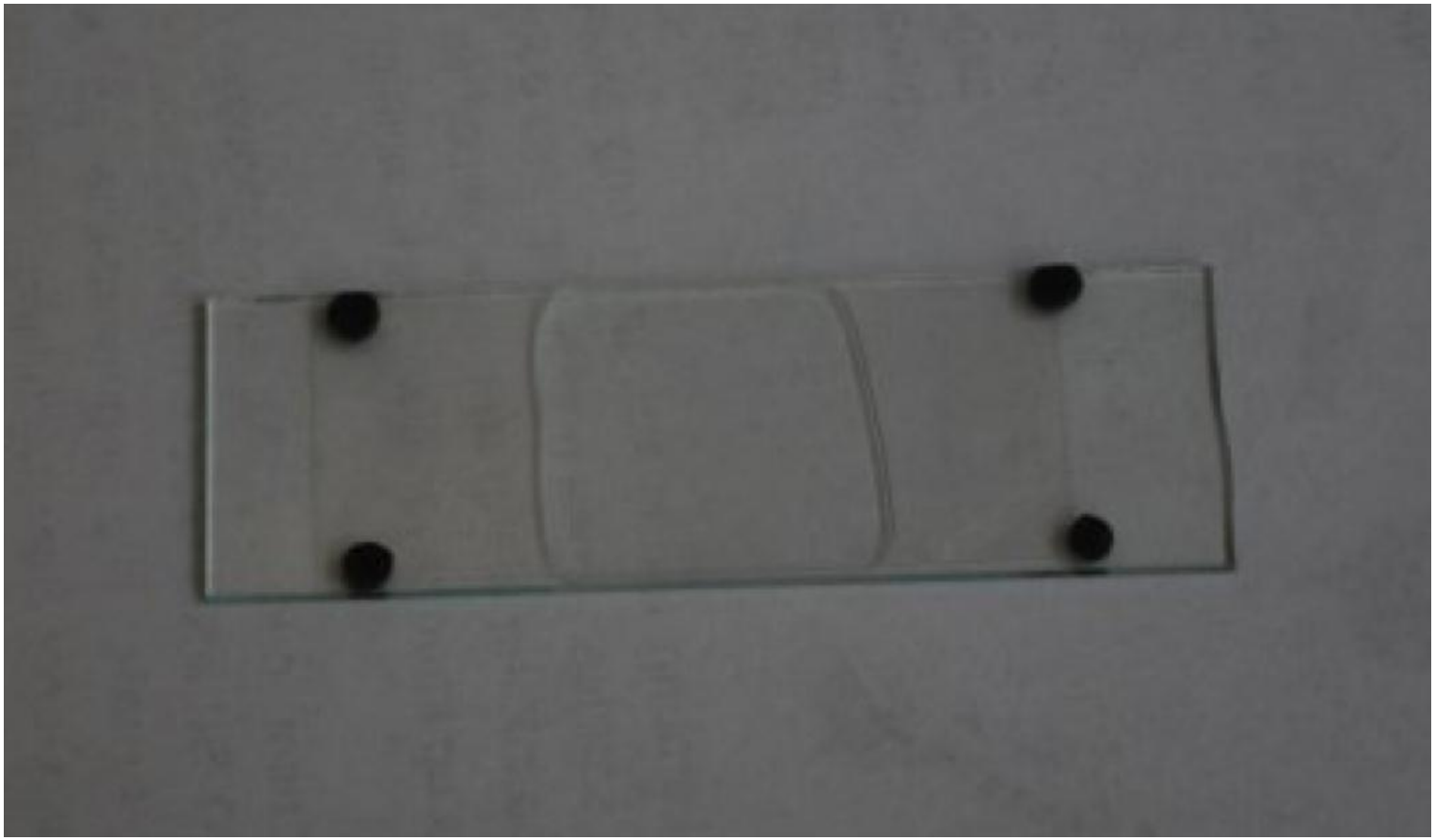
- **Negative control** – 100% filtered seawater
- **Positive control** – 20 mcg/ml Vincristine sulfate
- **Experimental group** - 4% Gracilaria salicornia extract

Experimental treatments



DATA COLLECTION

Clay Feet Preparation



Cell stages

A) 1-cell stage, (B) 2-cell stage, (C) 4-cell stage, (D) 8-cell stage, (E) 16-cell stage, and (F) 32-cell stage

		TREATMENT 1						TREATMENT 2						TREATMENT 3					
	R	Cell Stages						Cell Stages						Cell Stages					
Time 0		1	2	4	8	16	32	1	2	4	8	16	32	1	2	4	8	16	32
	1																		
	2																		
	3																		
	4																		
	5																		
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	5																		

Operational definition

Average cell stage – refers to the weighted mean number of cells per embryo.

Average cell stage
= (No. cells x cell $stage_1$ + No. cells x cell $stage_2$ + No. cells x cell $stage_{n...}$) /total number of cells

Operational definition

Time After Fertilization (minutes)	Number of Cells per Embryo						Average Cell Stage
	100% filtered seawater						
	1	2	4	8	16	32	
	No.	No.	No.	No.	No.	No.	
15	8	268	343	93	38	0	4.36

Average cell stage = $\dfrac{[(1x8) + (2x268) + (343x4) + (8x93) + (16x38)]}{8+268+343+93+38}$

$= \dfrac{8+536 + 1372 + 744 + 608}{750}$

$= \dfrac{3268}{750}$

= 4.36

Operational definition

Mitotic inhibition – based on the average cell stages at 165 minutes post administration of treatment with the following interpretation:

INTERPRETATION	AVERAGE CELL STAGE	SIGNIFICANCE
Sensitive	with an average cell stage of 1.0-2.0.	Effective inhibition of mitosis
Intermediate	with an average cell stage of 2.01-6.0.	Moderate inhibition of mitosis
Resistant	with an average cell stage of 6.01 and above.	Ineffective inhibition of mitosis

Data Analysis

SPSS version 16.0

- Analysis of Variance (ANOVA)
- Brown-Forsythe
- Multiple Pairwise Comparisons Test (Least Significant Difference)
- Chi-square test of homogeneity

P value of <0.05 is deemed as significant.

Results and Discussion

Summarized Results of 3 Trials among 100% filtered seawater, 4% *Gracilaria salicornia*, and 20 mcg/mL Vincristine sulfate at 165 minutes after fertilization

Treatment	Cell Stage												ACS
	1		2		4		8		16		32		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
100% filtered seawater	0	0	95	12.7	287	38.3	258	34.4	98	13.1	12	1.6	7.14
4% *G. salicornia*	724	96.5	22	2.9	4	0.5	0	0	0	0	0	0	1.05
20mcg/ml Vincristine	724	96.5	23	3.1	3	0.4	0	0	0	0	0	0	1.04

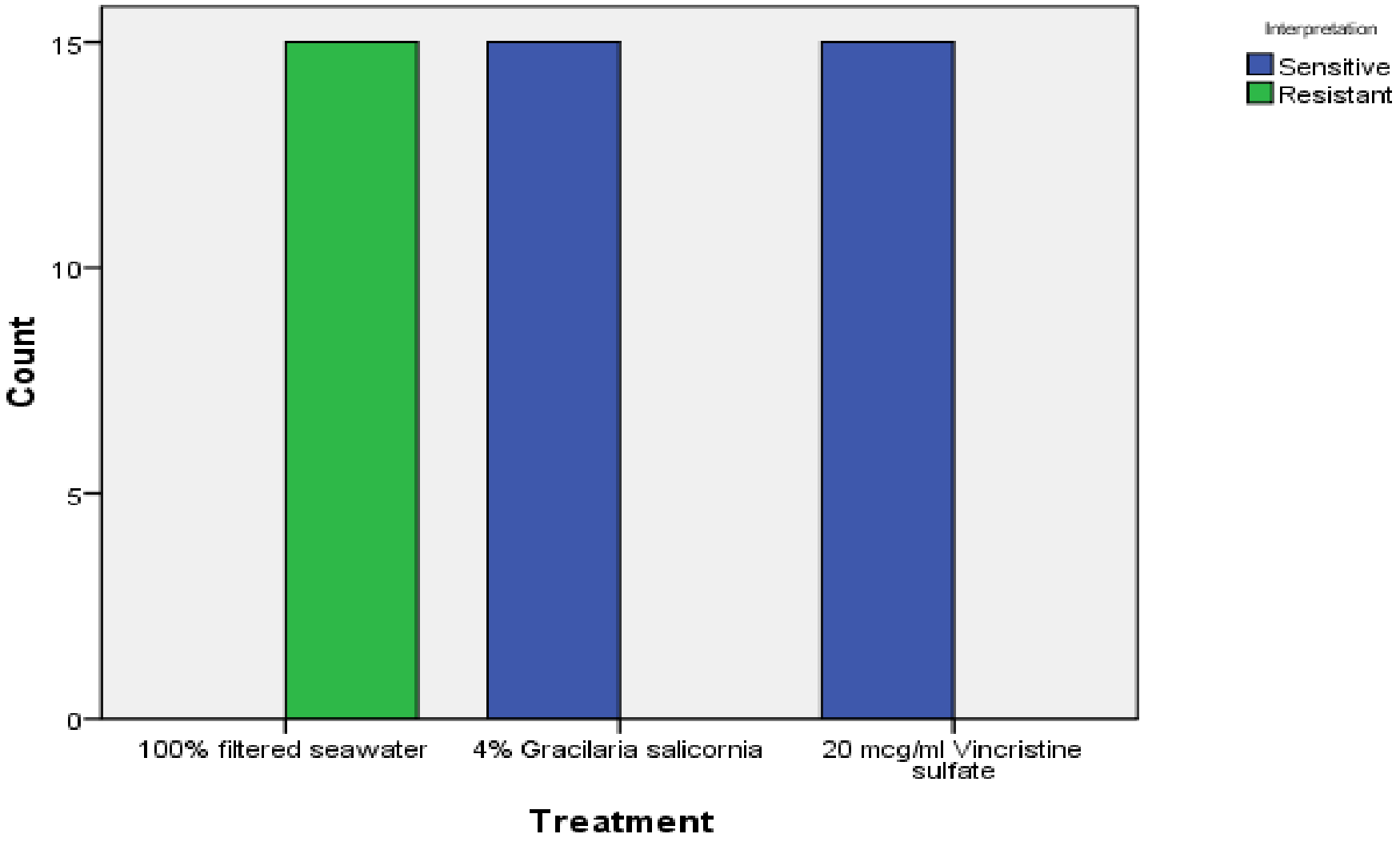
Average Cell Stage at 165 minutes among the three treatment groups

GROUP	Mean	Standard deviation	P value
100% Filtered Seawater	7.1387	.55752	.000
4% Gracilaria salicornia	1.0453	.02326	
20 mcg/ml Vincristine sulfate	1.0413	.03248	

MULTIPLE COMPARISON WITH LSD (Least Significant Difference)

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.
100% filtered seawater	4% Gracilaria salicornia	6.10933*	.11506	.000
	20 mcg/ml Vincristine sulfate	6.11200*	.11506	.000
4% Gracilaria salicornia	100% filtered seawater	-6.10933*	.11506	.000
	20 mcg/ml Vincristine sulfate	.00267	.11506	.982
20 mcg/ml Vincristine sulfate	100% filtered seawater	-6.11200*	.11506	.000
	4% Gracilaria salicornia	-.00267	.11506	.982

Comparison of the proportion of average cell stages that shows inhibition of mitosis defining sensitivity of the sea urchin embryos to the test substances.



Conclusion

4% *Gracilaria salicornia* possesses anti-mitotic activity which is comparable to Vincristine sulfate on the inhibition of mitosis of the fertilized *Tripneustus gratilla* embryos.

It is therefore a potential alternative to Vincristine Sulfate as an anti-mitotic agent.

Recommendations

- Isolation and characterization of the anti-mitotic compounds found in *G. salicornia*.
- Testing on vertebrate cells in future studies.
- Doing the experiment through a 12-month cycle.
- Screening for other unknown marine algae.

Thank you!

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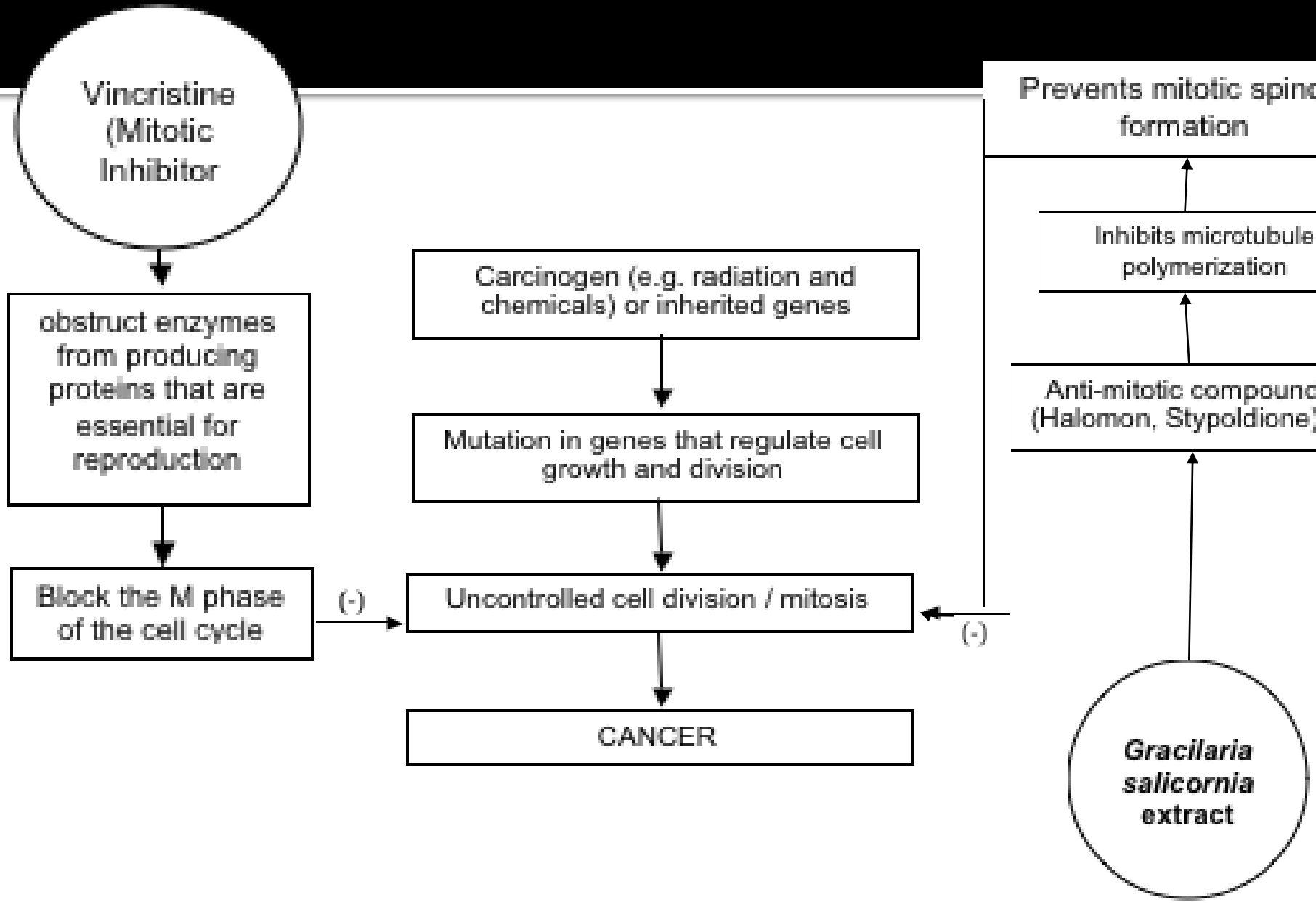
Conceptual framework



Summary

165 minutes post administration of the treatments:

4% Gracilaria salicornia - 1.05
20mcg/ml Vincristine - 1.04
100 % filtered seawater - 7.14

Significant difference : Between the 100 % filtered seawater to 4% Gracilaria salicornia and 20 mcg/ml Vincristine sulfate

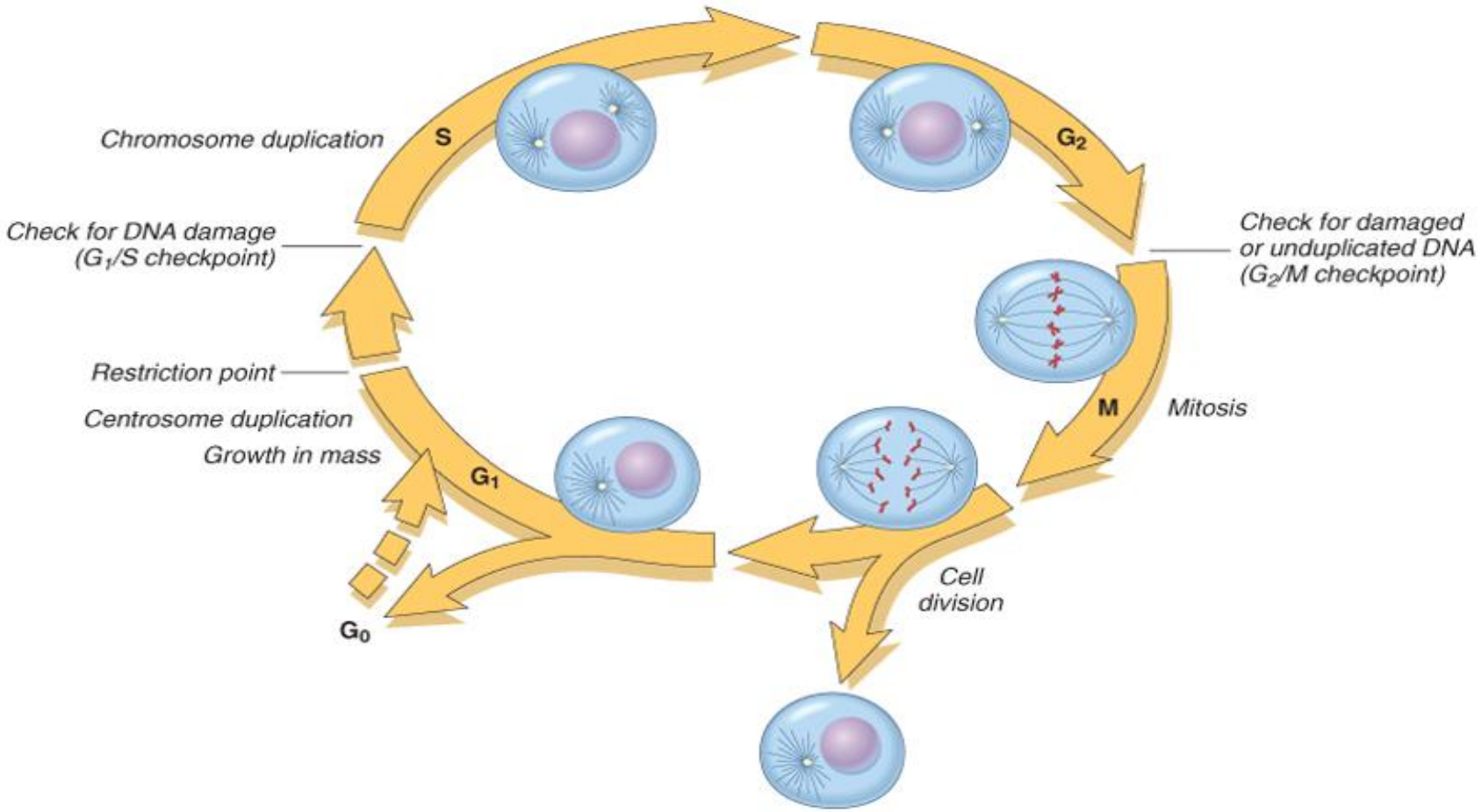
No significant difference: Between 4% Gracilaria salicornia and 20 mcg/ml Vincristine sulfate

Thus, *Gracilaria salicornia* is as effective as Vincristine, making it a possible alternative.

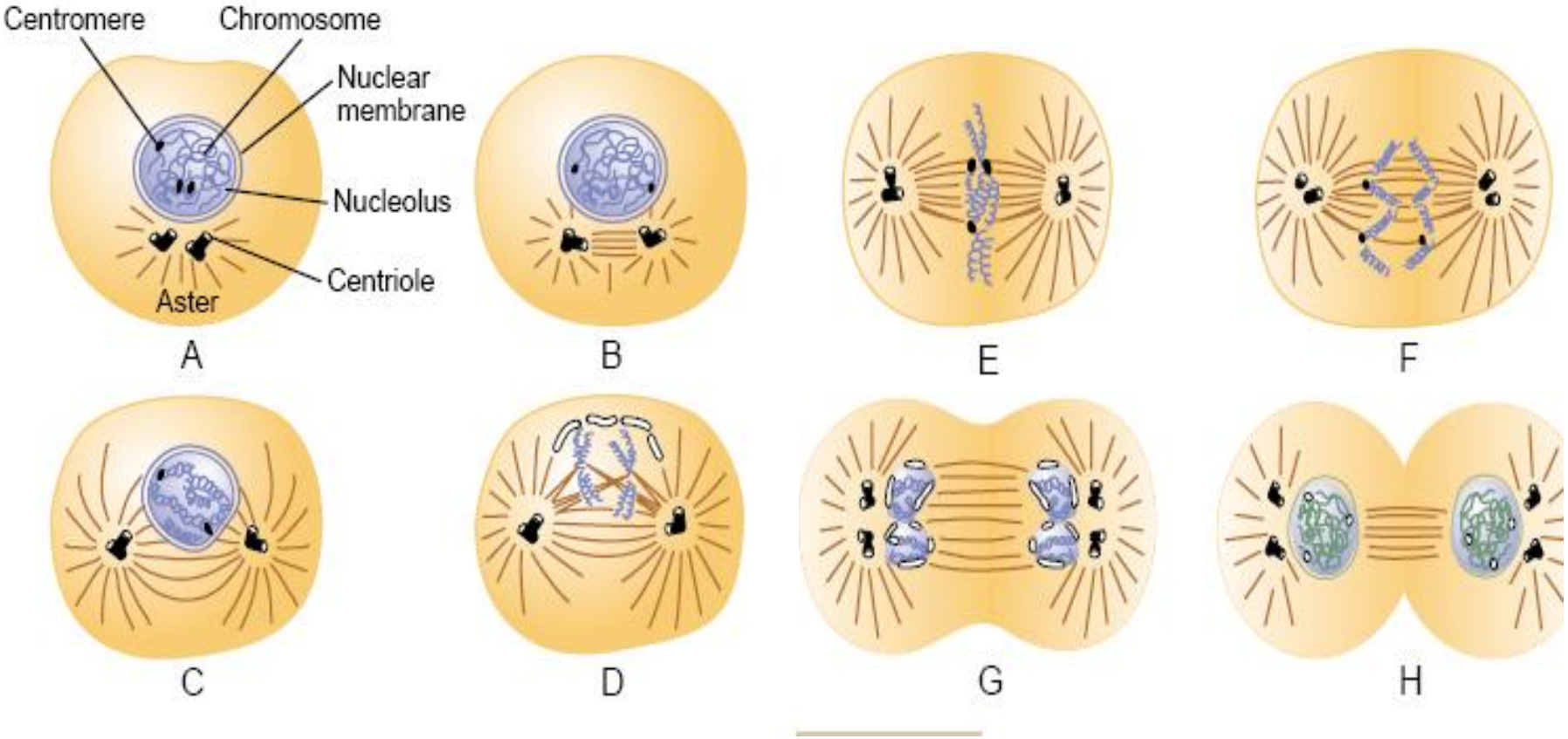
Some Anti-cancer Compounds from Marine Macroalgae

COMPOUND	SOURCE	ACTION
Stypoldione	*Stypodium zonale*	Inhibits microtubule polymerization and thereby prevents mitotic spindle formation
Iodinated nucleoside	*Hypnea valitiae*	A potent and specific inhibitor of adenosine kinase.
Curacin A	*Lynbya majuscula*	Repress the assembly of microtubules and binding of colchicines to tubulin
Caulerpenyne	*Caulerpa taxifolia*	Causes a shift in the S phase, blocking the G2 phase

Cell Cycle


Chromosome duplication
S
G2
Check for damaged
or unduplicated DNA
(G2/M checkpoint)
Check for DNA damage
(G1/S checkpoint)
Restriction point
Centrosome duplication
Growth in mass
M
Mitosis
G1
Cell
division
G0

Cell Cycle


Centromere
Chromosome
Nuclear membrane
Nucleolus
Centriole
Aster
A
B
E
F
C
D
G
H