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Anti-mitotic activity of *Citrus microcarpa* leaf extract on the *in vitro* development of Sea Urchin, *Tripneustes gratilla* embryo

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Abstract

Plant-derived compounds have played an important role in the development of several clinically useful anti-cancer agents. This study aims to determine the anti-mitotic activity of the ethanolic leaf extract of *Citrus microcarpa* to sea urchin embryos as a preliminary bioassay to determine its anti-cancer property. The mitotic inhibition activity of the plant extract to sea urchin embryos was observed in four (4) various treatment concentrations of the extract. The time interval of each developmental stage of sea urchin embryos treated with the different concentrations of the *C. microcarpa* extract was higher compared to negative control group. The lowest concentration (0.50%) of the plant extract showed the fastest mitotic activity compared to other concentrations. On the other hand, the highest concentration (2.00%) showed the slowest mitotic activity compared to other treatment concentrations. In addition, 0.50% concentration showed a comparable result with the positive control on the time interval during 2-cell stage. *C. microcarpa* leaf extract showed anti-mitotic activity to sea urchin embryos. The inhibition of sea urchin's proliferation in each developmental stage is dependent on the increase plant extract concentration. In addition, increasing concentration of the plant extract increased the time interval between developmental stages of sea urchin embryos. Results on phytochemical screening revealed that *C. microcarpa* contains the following phytochemicals: alkaloids, saponins, flavonoids, steroids and tannins. The antimitotic activity of the extract can be attributed to the phytochemicals present in the plant which can be a potent anti-cancer agent. Further studies for the isolation and identification of the bioactive compounds of these extracts should be undertaken.

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Introduction

Cancer is a major public health threat worldwide. It possesses burden on patient, families, and communities, sectors of the society and the national development of the country. Every year, 6 million worldwide suffer with cancer pain. Recent statistics showed that in the next twenty years, new cases of cancer would increase up to 70% in the world's total new cases (World Health Organization 2015).

Despite the current availability of a multiplicity of anticancer agents, there is a continuous search for new compounds that may be more effective and safe (Teicher, 2002). Plants that interfere with the normal progression of mitosis belong to the most successful chemotherapeutic compounds currently used for anti-cancer treatment (Schimdt, 2007).

Cancer is the uncontrolled growth of cells coupled with malignant behavior: invasion and metastasis. Cancer is thought to be caused by the interaction between genetic susceptibility and environmental toxins. In the broad sense, most chemotherapeutic drugs work by impairing mitosis (cell division), effectively targeting fast-dividing cells. As these drugs cause damage to cells they are termed cytotoxic and genotoxic (Naem *et al.,* 2009; Thenmozhi *et al.,* 2011). Some drugs cause cells to undergo apoptosis called "programmed cell death" (Naem *et al.*, 2009).

Eighty (80%) of the world's people depend on traditional medicine using plant extracts for their primary healthcare needs. The role of medicinal plants is very significant in the development of new drugs (WHO, 2002). Lotufo *et al.* (2005) stated that many drugs that are presently used chemotherapeutic agents were isolated from various species of plants or derived from a natural prototype. Plants that inhibit with the normal progression of mitosis belong to the most successful chemotherapeutic compounds currently used for anti-cancer treatment (Schimdt, 2007).

Some plants possess natural products or phytochemicals with medicinal value, such as steroids, tannins, alkaloids, flavonoids, and saponins. Tannins have anticancer activity and can be used in cancer prevention (Li and Wang, 2003). Alkaloids are toxic against cells of foreign organisms. Potential use in the elimination and reduction of human cancer cell lines. Saponins are cancer protective agents acting as antioxidants, & antimutagens (Nobori *et al.*, 1994).

Kalamansi, *Citrus microcarpa* is a citrus plant characterized by smooth and slightly spiny plant, growing to a height of 3 to 5 meters.

It is widely cultivated in the Philippines (Philippine Medicinal Plant). Some tribes of the Philippines use decoction of leaves to lower hypertension. Juice from partly roasted fruits used for coughs and colds (Olowa *et al.,* 2012). Craig (2002) stated that there are about 40 limonoids in citrus with limonin and nomilin being the principal ones. Limonoids possess the ability to inhibit tumor formation by stimulating the enzyme glutathione S-transferase (GST). Okwu (2008) affirmed that citrus plants of which kalamansi belongs contain the following phytochemicals: alkaloids, flavonoids, tannins, phenols and saponins.

Sea urchin egg assay is largely applied in investigation of anticancer properties of secondary metabolites.

The sea urchin eggs possess strong sensitivity against toxic agents which provide an important tool for discovery of drugs with anticancer potential (Lotufo, 2003).

There are factors that make this system suited for conducting a wide range of biological tests: straightforward artificial spawning, fertilization and rearing, rapid synchronous development, embryo optical transparency, and well understood embryogenesis. As a result, sea urchin embryos have been successfully used in studies of the effects of various antiproliferative agents (Nishioka, 2003).

This study aims to determine the anti-mitotic activity of Kalamansi, *Citrus microcarpa* leaf extract to the sea urchin, *Tripneustes gratilla* embryos.

Materials and methods

Collection and Preparation of Citrus microcarpa

Plant samples were collected from Cebu City. Plant leaves were washed with tap water and then rinsed with distilled water. These were then air dried for 48 hours at room temperature. Dried plant samples were chopped using a kitchen knife and pulverized using electric blender. The plant leaf powders were subjected for ethanolic extraction process.

Extraction of Plant Sample

Pulverized plant samples were placed in a glass container. The samples were soaked with ethanol in the ratio of one gram of samples is to one mL of ethanol (1:1) within 48 hours and then filtered. The resulting filtrates were then concentrated in a rotary evaporator.

Rotary Evaporation and Phytochemical Analysis

The plant extract was concentrated through rotary evaporation until it is semi-solid in form. The phytochemical screening was also conducted following the standard procedure as described by Harborne (1998). The ethanolic extract was evaluated for the qualitative determination of major phytochemical constituents such as alkaloids, flavonoids, tannins, saponins, steroids, anthraquinone, and cyanogenic glycosides.

Alkaloid

The detection of Alkaloids was carried out through extracting an equivalent of 10 grams of each plant extract that was recently evaporated on an evaporating dish to a syrupy consistency over a steam bath with 5 mL of 3 M HCl; and the filtrate was treated with Mayer's Wagner's reagent.

Flavonoids

The determination of flavonoids was done by taking another extract equivalent to 10 grams of the plant samples that was evaporated to incipient dryness over steam bath. Plant samples were then be added to a room temperature. The residue was defatted by treating it with 95% n-Hexane until the extract is almost colorless. Afterwards, the hexane extract was discarded. Its residue was taken up with 80% alcohol and finally added with hydrochloric acid. The appearance of color red within 10 minutes demonstrated positive test for flavonoids.

Tannins

The presence of tannins was determined through adding 10 grams of evaporated plant extract with 10 ml boiled distilled water, followed by the addition of 5 drops of 10% $FeCl_3$(ferric chloride) to the filtrate. Development of white precipitate was taken positive for the presence of tannins.

Saponins

Saponin content was confirmed by mixing the crude methanolic extracts with 10 mL distilled water. The extract was then shaken vigorously to record froth formation.

Steroids

The detection of steroids was done by extracting 20 grams of sample in 10 mL methanol. Five (5) mL of this methanolic extract was treated with 2 mL glacial acetic acid containing 1 drop of 5% $FeCl_3$solution. This solution was carefully transferred to the surface of 1 mL concentrated of H_2SO_4. The formation of reddish brown ring at the junction of two liquids was the indication of the presence of steroids.

Anthraquinone

Anthraquinone was detected by taking an amount of 10 grams of the plant samples and it was evaporated until it is almost dry over a steam bath. The residue was took up with 10 mL distilled water and filtered. The filtrate was extracted twice with 5 mL portions of benzene. Benzene extract was divided into portion, the other one served as control while the other was treated with 5 mL ammonia solution. The appearance of color pink in the lower alkaline layer indicated the presence of Anthraquinone.

Cyanogenic glycocides

Cyanogenic glycosides was tested through placing 2-5 grams of crushed plant material. It was moistened with enough distilled water and was then added with

a few drops of chloroform to enhance enzyme activity. One (1) mL of 1 % emulsion solution was added to ensure hydrolysis of glycoside. The test tubes was tightly covered with cork from which it was suspended with a piece of picrate paper that must not touch the inner side of the test tube. The tubes was warmed up at 35-40°C or kept at room temperature for three hours. Appearance of various shades of red within 15 minutes measures the relative concentration of cyanogenic glycoside.

Sea Urchin Bioassay

Collection of sea urchin.

Adult sea urchins, *Tripnuestes gratilla*, were collected from intertidal zone of Maribago, Lapu-Lapu City. It was then transferred into glass aquarium laid with *Sargassum sp.* and acclimatized in the laboratory for 48 hours. Collection was done during a new moon when gonad index of *T. gratilla* increases and peaks one day after the first quarter of the lunar cycle (Muthiga, 2005).

*Sexing and spawning induction***.**

Acclimatized sea urchins was rinsed with filtered sea water (FSW) to remove any debris. Ripe urchins was shaken vigorously to induce spawning enough to distinguish the male and female gametes, white and yellow-orange respectively. To trigger complete spawning of the remaining gametes, <1ml 0.55 KCl was injected into the coelomic cavity through the perioral membrane surrounding the lantern on the oral surface. Spawning male urchin was placed on a petri dish on ice. Collected sperm was transferred in a test tube and kept cold until before use. Spawning female urchin was inverted into a glass beaker with FSW just enough to submerge the gonophores. A sample of collected eggs was examined under the microscope (100x) to ensure that the eggs are mature and do not exhibit blebbing or lysis. Eggs were washed with FSW three times.

*Artificial fertilization***.**

Washed eggs were added with FSW 10% (v/v) suspension. Twenty-five (25µL) of sperm were diluted in 5mL FSW and mix thoroughly. Diluted sperm was added to 100mL of egg suspension and mix gently. Immediately, a drop of inseminated eggs was viewed under a microscope to observe the elevation of the fertilization envelope then dilute the culture in large amount of FSW.

*Determination of antimitotic activity***.**

Fertilized eggs were resuspended in a final volume of FSW to give a 0.5 to 1% (v/v) suspension. Zygotes were gently transferred into culture flasks and was added with the different concentrations of the plant extract and control. The actual concentrations of the extract which was used for the bioassay were determined after the range finding test. The flasks were placed on a shaker to allow small amount of water movement necessary for development of the culture. The different developmental stages of the zygotes were observed by examining a drop of fertilized eggs in a petri dish or depression slide on a microscope. There were four replicates for each treatment concentration and the control groups. The time interval for the embryonic development of *T. gratilla* treated with the different concentration of the plant extract and the control group from fertilized egg, 2-cell stage, 4-cell stage, 8-cell stage, 16-cell stage, and 32-cell stage of embryonic development was noted.

Results and discussion

The sea urchin bioassay is largely applied in investigations of anticancer properties of plant's secondary metabolites. The sea urchin egg possesses strong sensitivity against toxic agents and the development presents several peculiarities which provide an important tool for discovery of drugs with anticancer potential. The inhibition of mitoses can be related to different events of this process, such as DNA and RNA synthesis, protein synthesis, and mitotic spindle assembly. In some cases, it is possible to analyze these processes one at a time (Fusetani, 1987).

The present study evaluated the antimitotic activity of *Citrus microcarpa* ethanolic extract on the sea urchin embryo. Four different concentrations (0.5%, 1.0%,

1.5% and 2.0%) of *Citrus microcarpa* leaf extract served as the experimental group, positive control group treated with colchicine and negative control group uses seawater. The presence of a fertilization membrane in the embryos marks the start of fertilization as shown in Figure 1.b. Figure 2 shows the normal embryonic development of sea urchin embryos from zygote (1-cell stage) to 32-cell stage.

Table 1. Mean time interval of the early embryonic developmental stages of sea urchin eggs treated with the various concentrations of the *Citrus microcarpa* extract.

Treatments	Mean time interval of cleavage (minutes)				
	2-cell stage	4-cell stage	8-cell stage	16-cell stage	32-cell stage
Negative Control	48.75	9.0	22.00	14.75	19.50
Positive Control (Colchicine)	57.50	41.00	--	--	--
2.00%	96.50	61.75	--	--	--
1.50%	84.00	34.50	--	--	--
1.00%	63.50	30.50	52.50	19.25	81.50
0.50%	57.25	25.75	27.25	32.50	72.50

(--) - no cell division.

Table 2. Percentage of mitotic inhibition in sea urchin eggs treated with the various treatment concentrations of C. *microcarpa* extract and control groups at 1-hour time interval.

Treatment	Time	No. of fertilized eggs	1-cell stage	2-cell stage	4-cell stage	8-cell stage	16-cell stage	32-cell stage	% Mitotic Inhibition
Negative Control	1st hour	100	49	51					49.00
	2ndhour		9	25	50	15	1		9.00
	3rd hour		0	0	38	38	20	4	0.00
	4th hour		0	0	22	41	23	14	0.00
Positive Control	1st hour	100	91	9					91.90
	2nd hour		90	10					90.00
	3rd hour		86	12	2				86.00
	4th hour		85	15					85.00
2.00%	1st hour	100	96	4					96.00
	2nd hour		94	6					94.00
	3rd hour		89	11					89.00
	4th hour		73	20	7				73.00
1.50%	1st hour	100	91	9					91.00
	2nd hour		90	10					90.00
	3rd hour		85	15					85.00
	4th hour		70	20	10				70.00
1.00%	1st hour	100	86	12	2				86.00
	2nd hour		39	56	5				39.00
	3rd hour		0	15	54	27	4		0.00
	4th hour		0	5	9	35	39	12	0.00
0.50%	1st hour	100	92	8					92.00
	2nd hour		26	46	20	9			26.00
	3rd hour		0	0	8	22	47	23	0.00
	4th hour		0	0	3	15	32	50	0.00

Table 1 shows the mean time interval of cleavage in sea urchin eggs treated with the four (4) various concentrations of *C. microcarpa* extract and the control groups (positive and negative control). In the negative control, normal cell division was observed with the shorter time difference in every developmental stage which reaches up to 32-cell stage as compared to the various concentrations of the plant extract and the positive control (Colchicine). In the experimental group, 0.5% extract revealed the fastest rate of cell division among the treatment group, on the other hand, it shows slower rate of the time interval of mitotic activity compared to the negative control from 2-cell to 32-cell stage. Meanwhile, the highest concentration of plant extract (2.0%) revealed the slowest rate of mitosis that divides only until 4-cell stage.

Table 3. Phytochemicals present in the ethanoloic extract of *C. microcarpa*.

Plant sample	Alkaloids	Saponins	Flavonoids	Steroids	Tannins	Anthraquinone	Cyanogenic glycosides
Citrus microcarpa	++	+	+++	+++	+++	-	-

Legend: (-) absence; (+) less abundant; (++) moderate; (+++) very abundant.

The negative control revealed the fastest rate of cell division as compared to the various treatment concentrations of the plant extract while positive control (colchicine) was comparable to the result gathered in the 0.5% concentration in the 2-cell stage. Comparing the four different concentrations treated with *C. microcarpa* ethanolic extract, 0.5% concentration revealed the fastest rate of cell division while 2.0% concentration revealed the slowest rate of mitotic activity. Results also revealed that the sea urchin embryos treated with the two high concentrations (1.50% and 2.00%) of the plant extract manifest mitotic activity from 2-cell to 4-cell stage only contrary to the negative control which proliferate up to 32-cell stage.

This implies that the mitotic activity inhibition of the plant extract is concentration-dependent.

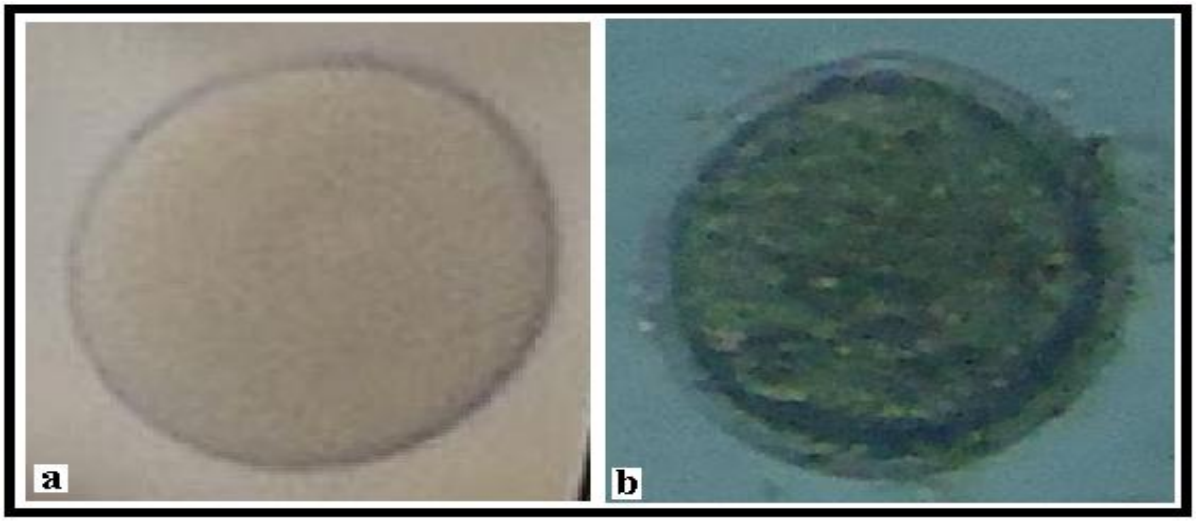

Fig. 1. *a*) Unfertilized egg; and *b*) fertilized egg of *T. gratilla*.

Result shows inhibition of mitotic activity in *T. gratilla* embryos treated with different concentrations of *C. microcarpa* extract. The delay of the development of each stage could be due to the disturbance of the cytoskeletal structures, which are critical for embryonic development. These structures establish the internal structure of the cell, which is essential for maintaining its normal functions (Semenova *et al.,* 2006).

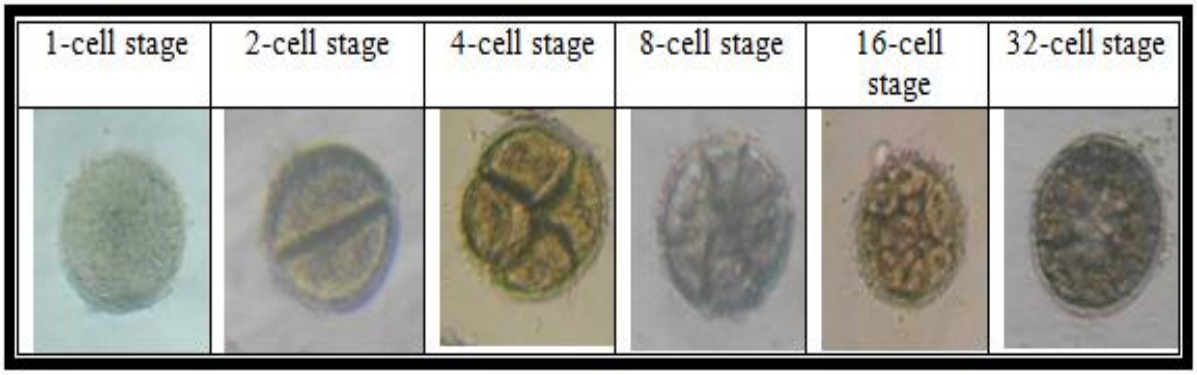

Fig. 2. The normal developmental stages of *T. gratilla* under negative control.

Results on Kruskal-Wallis Test reveal high significant differences, $p = 0.002$ on the time interval of the various mitotic activities or cell-stage development (2-cell stage, 4-cells stage, 8-cell stage, 16-cell stage and 32-cell stage) treated with the various concentrations of the *C. microcarpa* extract and the control groups (Figures 3-7). The delay and inhibition of the cell division could be due to the disturbance of the cytoskeletal structures, which are critical for embryonic development and essential for maintaining its normal functions. Semenova *et al.* (2006) also stated that the disruption of the cell division could be due to the disturbance of the tubulin. Tubulin is a major protein in microtubule, cell organelle which play significant role in mitosis specifically in the formation of mitotic spindle and separation of chromosomes during anaphase. Thus, deactivating tubulin in rapidly proliferating tumor cells is an effective way for cancer therapy.

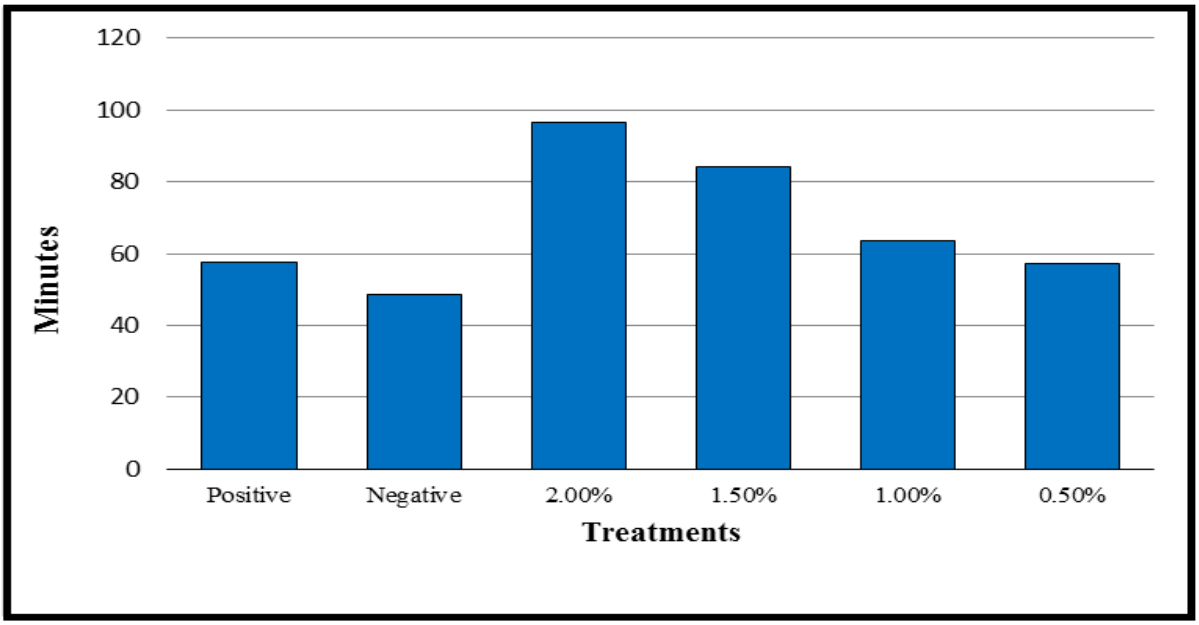

Fig. 3. Mean time (minutes) interval of the early embryonic developmental stages of sea urchin eggs at 2-cell stage treated with the various concentrations of the *Citrus microcarpa* extract and the control groups.

Table 2 shows the percentage of mitotic inhibition of sea urchin egg cells treated with the various treatment concentrations of *C. microcarpa* leaf extract and control groups at 1-hour time interval. After one hour of exposure, 51% of sea urchin eggs in the negative control group undergo mitotic activity in 2- cell stage, on the otherhand, the four various treatment concentrations, 0.50%, 1.00%, 1.50%, and 2.00% of the extract revealed very low percentage of mitotic activity which is equal to 8%, 12%, 9%, and 4%,

respectively. After 2 hours of exposure, the negative control showed the fastest rate (91%) of mitotic activity which reaches up to 16-cell stage and 50% of the dividing cells are in the 4-cell stage. However, the highest concentration (2.00%) of the plant extract revealed very low mitotic activity (4%) and reaches up to 2-cell stage only.

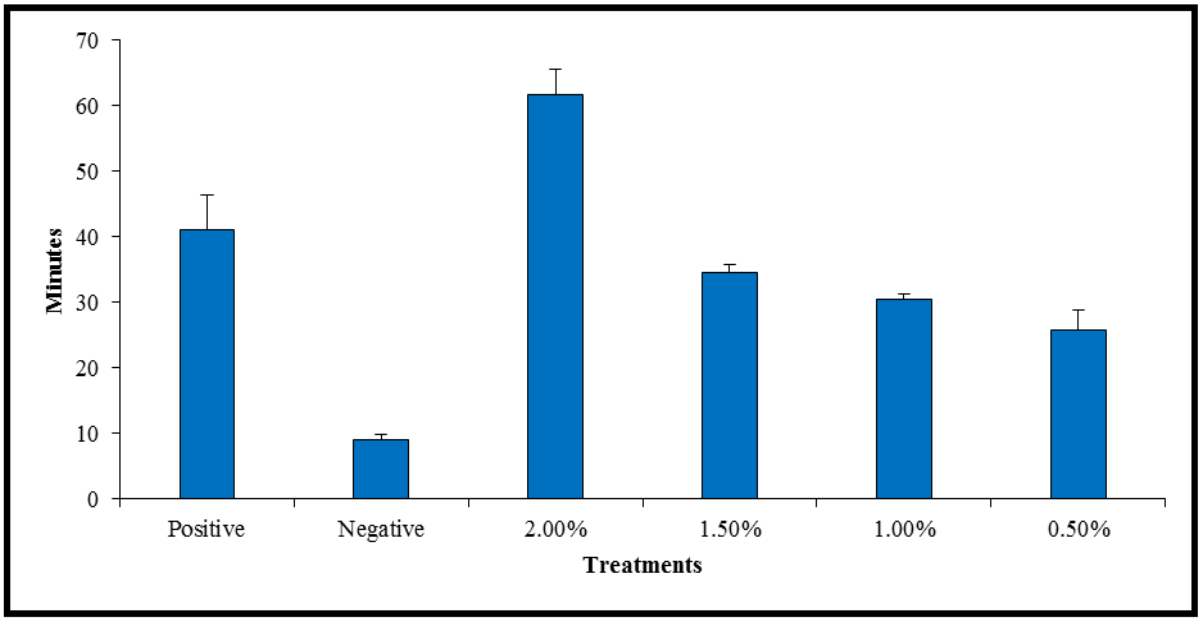

Fig. 4. Mean time (minutes) interval of the early embryonic developmental stages of sea urchin eggs at 4-cell stage treated with the various concentrations of the *Citrus microcarpa* extract and the control groups.

After three and four hours of exposure, the negative control showed 100% mitotic activity which reaches up to 32-cell stage. Meanwhile, the highest concentration (2%) of the treatment group exposed to leaf extract remains the lowest rate of mitotic activity (11% and 27% respectively and reaches up to 4-cell stage only. Kruskal-Wallis Test revealed significant increase, $p < 0.05$ on the mitotic inhibition in sea urchin eggs treated with the various treatment concentrations of C. *microcarpa* extract and control groups at 1-hour time interval. Comparing the four different concentrations treated with the plant extract, the lowest concentration (0.50 %) manifested the highest percentage of mitotic activity, on the otherhand, and the highest concentration (2.00%) revealed the lowest percentage of mitotic activity which reaches up to 4-cell stage only within four hours of exposure.

It indicates that the plant extract possess anti-mitotic activity to sea urchin embryos. Moreover, increasing concentration of the plant extract manifest a significant decreased of the mitotic activity of the sea urchin embryos which means that the mitotic inhibition is concentration-dependent.

Phytochemical analysis of C. *microcarpa* showed that its leaves contain alkaloids, saponins, flavonoids, steroids and tannins (Table 3).

The antimitotic functions of the phytochemicals present in the plants that mainly interacts with the disruption of microtubule and cell cycle.

Alkaloids are potential for the elimination and reduction of human cancer cell lines (Okwu, 2004). It also acts as antioxidant, anti-depressant and anti-inflammatory, and bactericidal.

In addition, Moudi *et al.* (2013) stated that alkaloids bind to the building blocks of a protein called tubulin, during cell division and inhibiting its formation. Saponins are cancer protective agents acting as antioxidants, and antimutagens (Nobori *et al.*, 1994).

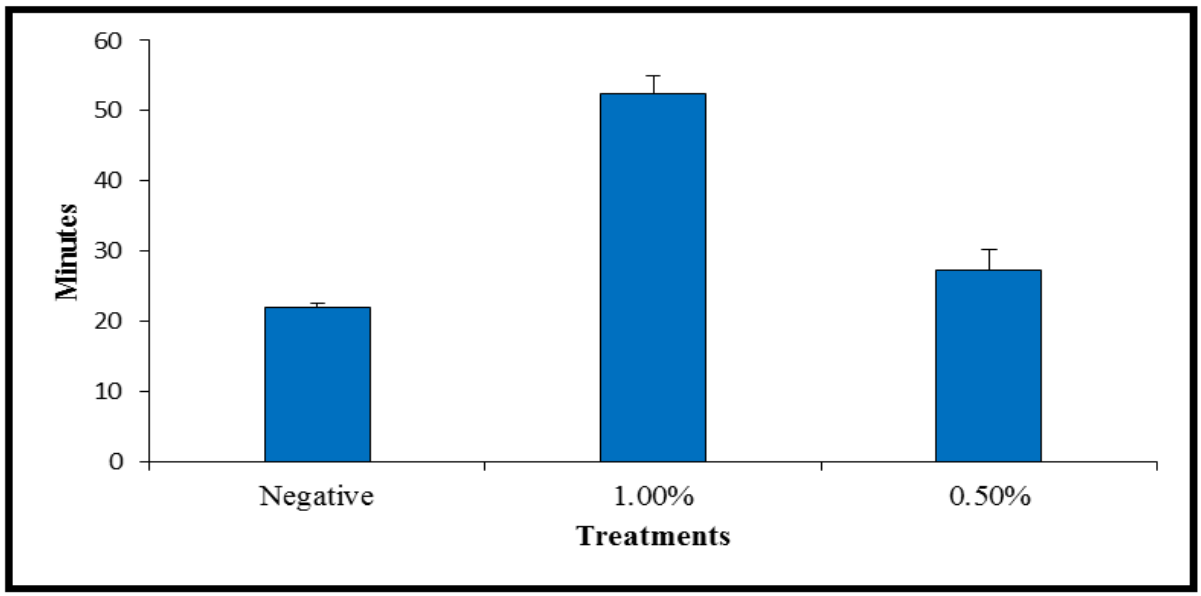

Fig. 5. Mean time interval of the early embryonic developmental stages of sea urchin eggs at 8-cell stage treated with the various concentrations of the *Citrus microcarpa* extract.

The presence of saponins supports the fact that C. *microcarpa* leaf has cytotoxic effects such as permealization of the intestine as saponins are cytotoxic (Okwu, 2001). Flavonoids interfere with cyclin-dependent cell cycle regulation and interact with drug transport (Halliwel, 2007). Ahmed *et al.* (2015) stated that flavonoids inhibit the proliferation of cell lines and demonstrated strong cytotoxicity towards colon cancer cells. Steroids block the G2/M phase of cell cycle, induce apoptosis, and change of Ca^{2+} distribution that triggers the cytoplasmic event breakdown in somatic cells (Hoffmannova *et al.* (2012). Tannins exhibit chemical structure that possess anticancer activities through enhancement of the host-defense potential (Miyamoto, 1999). Moreover, tannins are responsible for the stimulation of phagocytic cells, host mediated tumor activity and anti-infective actions in humans (Bandaranayake, 2002); and also possess anticancer activity and can be used in cancer prevention (Li and Wang, 2003).

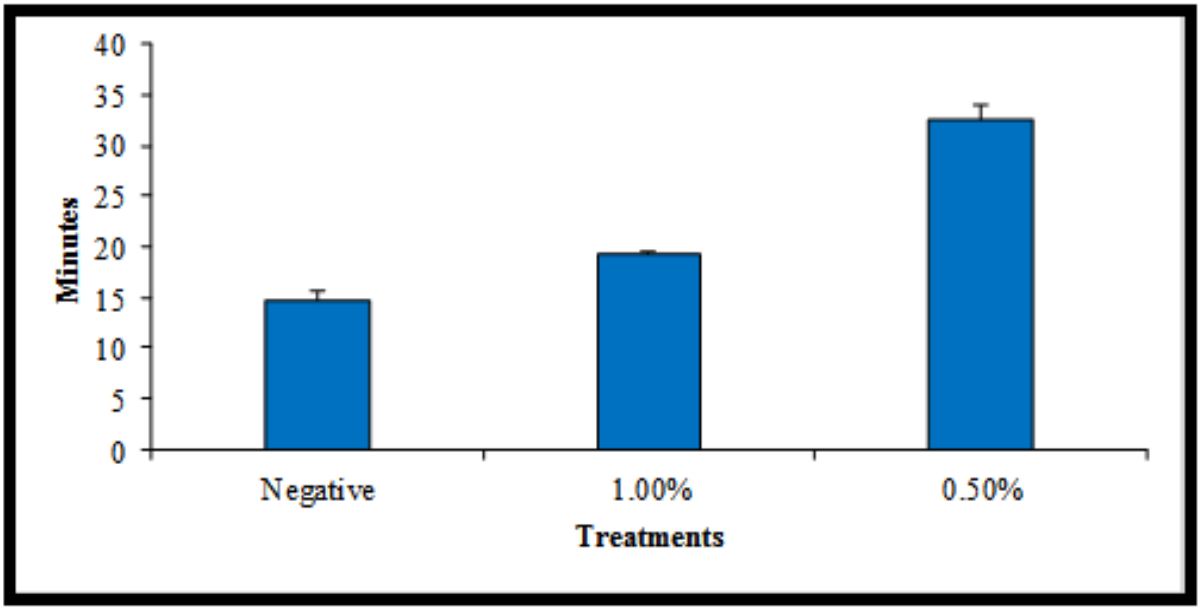

Fig. 6. Mean time interval of the early embryonic developmental stages of sea urchin eggs at 16-cell stage treated with the various concentrations of the *Citrus microcarpa* extract.

Sea urchin embryogenesis was known to have well-defined developmental stages that demand microtubule function (Raff *et al.,* 1971). Microtubules are extremely important cellular entity with a crucial role in shape maintenance, cell motility, intracellular transport and cell division (Bray, 2001). Moreover, they contain heterodimeric tubulin subunits that will form into multi-subunit microtubules when it undergoes polymerization (Sconzo *et al.,* 1995). These are the possible structures that can be affected by the antimitotic activity of *Citrus microcarpa* extract.

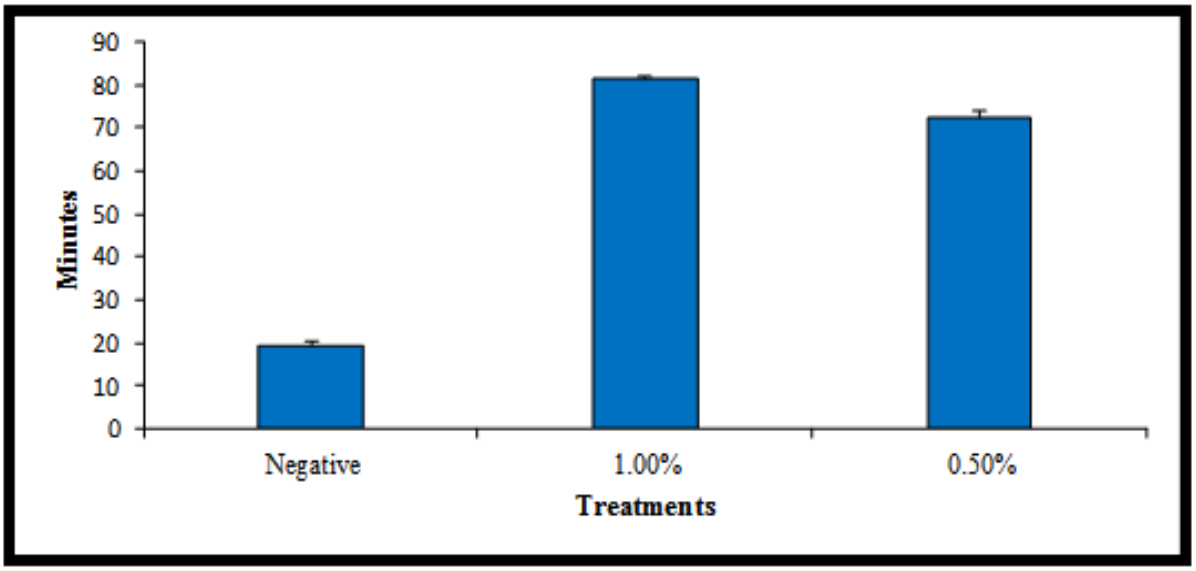

Fig. 7. Mean time interval of the early embryonic developmental stages of sea urchin eggs at 32-cell stage treated with the various concentrations of the *Citrus microcarpa* extract.

The dynamic characteristic interfered or inhibited in the cellular processes, not only on the mitotic spindle formation but also the cyclin-dependent kinases. Cyclin-independent kinase (CDK) is a protein kinase that needs another subunit (cyclin) that supplies domains for enzymatic processes. CDKs have significant roles in cell division and adjust the transcription on its intracellular and extracellular cells (Malumbres, 2014). Deregulation of CDKs pathway will affect the cell cycle as it regulates the transition through the G1 phase of the mitotic cycle. Thus, chemical compounds that target CDKs are good indicators for an antimitotic agent (Liu *et al.,* 2009).

Much earlier studies using sea urchin fertilization assay reported strong cytotoxicity of taxol due to inhibition of microtubule depolymerization in vitro and in vivo on sea urchin eggs during fertilization (Schiff *et al.*, 1980) and cannabinoids by preventing the initiation of acrosome reaction thereby reducing the fertilizing capacity of sperm (Schuel *et al.*, 1991).

Sea urchin fertilization was also inhibited by plant lectins that bind to the egg and sperm of the sea urchin (Macedo *et al.*, 2007). Recently, the leaf extract of *Carica papaya* which contains flavonoids, alkaloids, phenolic compounds and cynogenetic compounds exhibited antimitotic activity on cell proliferation of sea urchin embryos (Gutierrez, 2016). The sea urchin egg development possessed some peculiarities, which allow a preliminary interpretation on mode of action. The sea urchin cell cycle is highly abbreviated, essentially cycling from S (DNA synthesis) to M (mitosis) and S with no G1 and a relatively short G2 phase (Jacobs & Wilson, 1986). The inhibition of the first cleavage of the sea urchin egg development is related to DNA and=or protein synthesis or microtubule assembling, once RNA synthesis is very slow or absent after fertilization (Gross *et al.*, 1964; Brandshort, 1985).

Plant products such *Oroxylum indicum*, *Moringa oleifera* and *Aegles marmelos* (Lotufo *et al.*, 2005);

Harpalyce brasiliana (Militao *et al.*, 2007); and *Amburana cearensis* (Lotufo *et al.*, 2003) which showed antimitotic effects to sea urchin embryo also found effective in the prevention of cell proliferation of cancer cells.

Conclusions

The study on the anti-mitotic activity of *Citrus microcarpa* leaf extract was determined using sea urchin bioassay. Results revealed that *C. microcarpa* leaves manifest mitotic activity inhibition against sea urchin embryos which is manifested by a significant increase of the time interval between developmental stages of sea urchin embryos exposed to various concentrations of the plant extract as compared to the control groups. In addition, as the concentration of the extract increases, mitotic arrest is prevalent of which cell division ended up at 4-cell stage only in 1.50% and 2.00% concentrations.

It is also noted that mitotic activity inhibition is dependent on the concentration of the plant extract. Moreover, Kruskal-Wallis Test revealed significant increase, $p < 0.05$ on the mitotic inhibition in sea urchin eggs treated with the various treatment concentrations of C. *microcarpa* extract and control groups at 1-hour time interval. Results on phytochemical screening revealed that *C. microcarpa* contains the following phytochemicals: alkaloids, saponins, flavonoids, steroids and tannins.

The antimitotic activity of the extract can be attributed to the phytochemicals present in the plant which are known to possess anti-tumor and anti-cancer properties. Results suggest that anti-mitotic activity of *C. microcarpa was* due to the phytochemical components which cause an inhibition of microtubule dynamic and interference of cyclin-dependent cell cycle regulations. *C. microcarpa therefore* contains anti-mitotic constituents which inhibit from cell proliferation and can be associated on its cytotoxic effect and a potential as anti-cancer agent. Further studies be done to validate the results and elucidate mechanism of action of the bioactive components present in the extracts.

References

Costa T, Vieria R, Bizzo H, Silveria D, Gimenes M. 2010. Potential of secondary Metabolites 131-165.

Craig E. 2002. Phytochemical Guardians of Our Health, Journal of American Dietetic Association **97(2),** 199-204.

Cragg, Newman. 2004. Plants as a Source of Anti-cancer Agents, in Ethnopharmacology, in Encyclopedia of Life Support Systems (EOLSS), Developed under the Auspices of the UNESCO, Eolss Publishers, Oxford, UK.

Grant WF. 1982. Chromosome aberration assays in *Allium*. A report of the USEPA Gene-Tox program. Mut Res **99,** 273-291.

Gutierrez PM. 2016. Antimitotic activity of Carica papaya in the in vitro development of sea urchin, Tripneustes gratilla embryo. International Research Journal of Biological Sciences **5(6),** 12-17.

Harborne JB. 1998. Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis.5th Edition, Chapman and Hall Ltd, London, 21-72.

Hausteen. 1983. Flavonoids, a class of natural products of high pharmacological potency. Biochemical Pharmacology **32,** 1141-1148.

Liu Henkel. 2002: Traditional Chineese medicine (TCM): are polyphenols and saponins the key ingredients triggering biological activities. Current Medicinal Chemistry **9,** 1483-1485.

Macedo NMR, Costa-Lotufo LV, Pessoa C, Moraes MO, Bonfim LR, Ramos MV. 2007. Inhibition of Sea Urchin Fertilization by Plant Lectins. Protein and Peptide Letters **14,** 886-893.

Militão GC, Pinheiro SM, Dantas IN, Pessoa C, Moraes MO, Costa-Lotufo LV, Lima MA, Silveira ER. 2007. Bioassay-guided fractionation of

pterocarpans from roots of Harpalycebrasiliana Benth. Science Direct 6687-6691.

Munro M, Blunt J, Dumdei E, Hickford S, Lill R, Li S. 1999. The Discovery and **development** of marine Compounds with Pharmaceutical Potential. J. Biotech **70,** 15-25.

Naem Q, Bashir AC, Altaf D. 2009. Phytochemical study of aerial parts of Lantana camara for the pharmacological active compounds. Journal of Applied Pharmaceutical Science **1(1),** 19-26.

Nishioka D, Marcell V, Cunningham M, Khan M, Von Hoff DD, Izbicka E. 2003. The use of early sea urchin embryos in anticancer drug testing. Methods in Molecular Medicine **85,** 265.

Nobori. 1994. Deletion of the cyclin-dependent kinase-4 inhibitor gene inmultiple human cancers. Nature **368(6473)**, 753-756.

Okwu D. 2008. Citrus Fruits: A Rich of Phytochemicals and their roles in Human health. International Journal of Chemical Science **6(2),** 451-471.

Olowa L, Torres MJ, Aranico EC, Demayo CG. 2012. Medicinal Plants Used by the Higaonon Tribe of Rogongon, Iligan City, Mindanao, Philippines. Advances in Environmental Biology **6(4),** 1442-1449.

Powis G. 1983. Dose-dependent metabolism, therapeutic effect, and toxicity of anticancer drugs in man. Drug Metabolism Reviews **14**, 1145-1163.

Prakash O, Kumar A, Kumar P, Ajeet. 2013. Anticancer Potential of Plants and Natural Products: A Review. American Journal of Pharmacological Sciences **1(6)**, 104-115.

Roowi S. 2008. Tropical citrus antioxidants and catabolism of phenolics in green tea, coffee, cocoa and orange juice/PhD thesis/University of Glasgow.

Russo G. 2007. Ins and outs of dietary phytochemicals in cancer chemoprevention. Biochemical Pharmacology **74,** 533-544.

Schiff PB, Horwitz SB. 1980. Taxol stabilizes microtubules in mouse fibroblast cells Proceedings of the National Academy of Science **77,** 156l 1565.

Schuel H, Berkery D, Schuel R, Chang MC, Zimmerman AM, Zimmerman S. 1991. Reduction of the fertilizing capacity of sea urchin sperm by cannabinoids derived from marijuana. Molecular Reproduction and Development **29(1),** 51-9.

Semenova M, Kiselyov A, Semenov V. 2005. Sea urchin embryo as a model organism for the rapid functional screening of tubulin modulators. Research Report. Bio Techniques 40.

Shweta S, Khadabadi S, Tapadiya Ganesh G. 2012. In vitro Evaluation of Antimitotic, Ant proliferative, DNA fragmentation and anticancer activity of Chloroform and Ethanol extracts of *Revia hypocrateriformis* Asian Pacific Journal of Tropical Disease. S503-S508.

Smaka-Kinel V, Stegnar P, Lovka M, Toman MJ. 1996. The evaluation of waste, surface and ground water quality using the *Allium* test procedure. Mutation Research **368,** 171-179.

Teicher BA. 2002. Tumor models in cancer research. Humana Press. New Jersey.

Thenmozhi A, Nagalakshmi A, Mahadeva Rao US. 2011. Study of Cytotoxic and Antimitotic Activities of *Solanumnigrum*by Using *Allium* cepa Root Tip Assay and Cancer Chemo preventive Activity Using MCF-7- Human Mammary Gland Breast Adenocarcinoma Cell Lines. International Journal of Science & Technology **1(2)**.

Wei L. 2008. Antimicrobial properties of tropical plants against 12 pathogenic bacteria isolated from

aquatic organisms. African Journal of Biotechnology **7(13),** 2275-2278.

Wei L, Najlah M. 2009. Antimicrobial Property of 2-Hydroxypropane-1, 2, 3- Tricarboxylic Acid Isolated from Citrus microcarpa Extract /Agricultural Sciences in China 8**(7),** 880-886.

WHO. Traditional Medicines Strategy. 2000. WHO/EDM/TRM/200.1, 2002-2005.