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Isolation of garcinone E from *Garcinia mangostana* Linn and its cytotoxic effect on sp2/0 cell lines

Suchitra Ramesh, Moghana Priya K and S Prabhu

Abstract
Research around the world is being aimed at identifying new and effective therapeutic products to serve as a substitute for the substances that are currently being used in various industries. It is well-recognized that consumption of fruits and vegetables can reduce the incidence of degenerative diseases including cancer, heart diseases, inflammation and inflammation. Many tropical plants have biological activities with potential therapeutic applications.
It has been found that Garcinone E, a xanthone derivative found in the hulls of *Garcinia mangostana* has potent anti-bacterial, anti-inflammatory and anti-cancer effects. This study focused on isolating Garcinone E from the pericarp of mangosteen using Soxhlet Apparatus with two solvents ethanol and ethyl acetate and further subjecting it to column chromatography and characterization using UV spectrophotometer and HPLC. The ethanolic extract gave a yield of 31.9%, showed a peak of 253nm and had a retention time of 2.78 mins which corresponds to the standard of Garcinone E. The cytotoxic effect of the isolated compound was evaluated *invitro* on mouse myeloma cells using Tryphan Blue dye exclusion method and MTT assay which showed that Garcinone E has potent cytotoxic effect on the Sp2/0 cell lines at 48hrs incubation time with 250µl volume of Garcinone E. Further studies could be focussed on studying the mechanism of action on the cells.

Keywords: Garcinia *mangostana*, Xanthones, Garcinone E, Anti-cancer properties, Cancerous cell lines

1. Introduction
Nutraceutical is defined as any substance that is food of part of food that provides medical or health benefits, for the prevention and treatment of diseases (De Felice *et al*., 1995). It includes a broad range of categories such as functional foods, dietary supplements and herbal products (Radhika *et al.*, 2011). Today, many compounds or phytochemicals in plants, especially in fruits have been associated with numerous health benefits (Lachance *et al.*, 2011). These are therefore used as ingredients in many nutraceuticals and pharmaceutical products.
Garcinia mangostana Linn is a tropical fruit available in Southeast Asia like Indonesia, Malaysia, Sri Lanka, Philippines and Thailand. It belongs to the family of Guttiferae and is named "the queen of fruits". It is also known as mangosteen. The edible portion of the fruit comprises only 25% of the total volume, whereas the remainder is a tough, bitter pericarp which exudes a yellow resin (hence the term xanthone or yellow in Greek). The mangosteen fruit is shown in Fig 1.

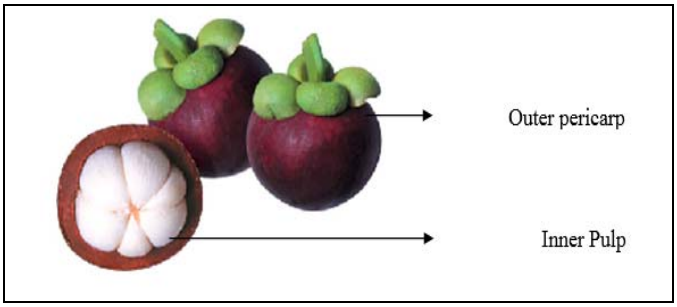


Fig 1: *Garcinia mangostana* fruits (Pieris *et al*., 1982)

The mangosteen rind, leaves and bark have been used as folk medicine for thousands of years. The leaves are used by some natives in teas to treat diarrhoea, dysentery and fever. It is also known that the concentrates of mangosteen bark can be used for genitor-urinary afflictions and stomatosis. People have used the pericarp (peel, hull, rind or ripe) of GML as a traditional medicine for the treatment of abdominal pain, infected wounds, inflammation, skin infections, suppuration, and chronic ulcer. Table 1 represents the traditional medicinal properties of *Garcinia mangostana*.

Table 1: Traditional medicinal properties of *Garcinia mangostana* (Jose *et al*., 2008) [7]

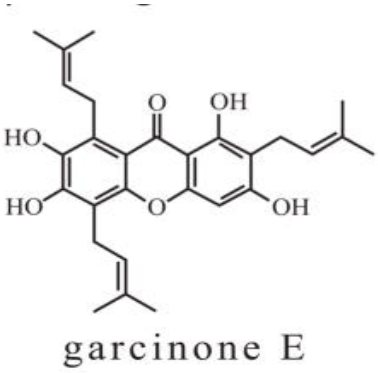
Illness	Reference
Haemorrhoids	Pierce (2003)
Food allergies	Pierce (2003)
Skin infections	Mahabusarakam *et al*. (1987), Pierce (2003) and Jinsart *et al*. (1992)
Tuberculosis	Harbone *et al.* (1999) and Suksamrarn *etal*. (2006)
Inflammation	Saralamp *et al.* (1996), Chairungsrilerd *etal*. (1996a,b) and Harbone *et al.* (1999)
Ulcers	Harbone *et al*. (1999) and Hasegawa *et al*. (1996)
Micosis	Saralamp *et al*. (1996) and Harbone *et al*. (1999)
Gonorrhea, cystitis and urethra suppuration	Garnett and Sturton (1932), Morton (1987) and Moongkarndi *et al*. (2004a)
Mouth aphthae	Caius (2003)
Fever	Caius (2003), Morton (1987) and Yates and Stout (1958)
Amoebic dysentery	Caius (2003) and Morton (1987)
Thrush	Morton (1987)
Abdominal pain	Moongkarndi *et al*. (2004a)
Suppuration	Moongkarndi *et al*. (2004a)
Leucorrhoea	Moongkarndi *et al.* (2004a)
Cholera	Sen *et al*. (1980a)
Convulsants	Malawska (2005)

Two major groups of phytochemicals in the mangosteen fruits are the xanthones and phenolics. Xanthones or Xanthen-9H-ones are secondary metabolites found in some higher plant families, fungi and lichens and they comprise an important class of heterocycles. Their taxonomic importance in such families and their pharmacological properties has aroused great interest not only for the chemosystematic investigations but also from pharmacological point of view like anti-malarial, anti-bacterial, anti-fungal, anti-viral, anti-inflammatory, anti-allergy, anti-oxidant and anti-tumoral properties.

Xanthones have been isolated from the pericarp, whole fruit, bark and leaves of GML. Several studies have shown that xanthones obtained from mangosteen fruits has remarkable biological activities. 50 xanthones have been isolated from the pericarp of mangosteen fruits out of which α-, β-, γ-mangostins, Garcinone E, 8-deoxygartanin and Gartanin are the most studied xanthones. A high content of xanthones has been detected in the pericarp when compared with the white aril part of the fruits.

Garcinone E is one of the xanthone derivatives present in *Garcinia mangostana* Linn. *Ho et al.,* (2002) [2] evaluated that Garcinone E has a potent cytotoxic effect against hepatocellular carcinoma cell lines and lung carcinoma cell lines. Gastric carcinoma cell lines are also sensitive to the anti-proliferative effect of Garcinone E (Huang *et al.,* 2002) [2]. Garcinone E was found to exhibit a very broad spectrum of dose- and time- dependent cytotoxic effects against various cancer cell lines.

Fig 2 represents the chemical structure of Garcinone E and Table 2 represents the chemical taxonomy of Garcinone E.



garcinone E

Fig 2: Chemical structure of Garcinone E

Molecular formula: $C_{28}H_{32}O$
Molecular weight: 464.6

Table 2: Chemical Taxonamy of Garcinone E

Kingdom	Organic Compounds
Super Class	Aromatic Heteropolycyclic Compounds
Class	Benzopyrans
Sub Class	Xanthenes
Other Descriptors	• Benzopyrans • Xanthenes
Substituents	• 1,2 Diphenol • Chromone • Isoprene • Phenol • Phenol Derivative • Pyran • Pyranone • Resorcinol
Direct Parent	Xanthones

Cancer is one of the leading causes of mortality worldwide. On a yearly basis in US, 0.5% of the population is diagnosed with cancer. Despite improved imaging and molecular diagnostic techniques, cancer continues to affect millions of people globally. An efficient molecule to treat cancer is inevitable and explorations to develop new entities are going on. However, nature has long been shown an excellent and reliable source of new drugs, including anti-cancer drugs. Plants are playing an important role as a source of anti-cancer drugs and the mechanism of interaction between many phytochemicals and cancer cells has been studied extensively (Vithya *et al*, 2012).

Treatment of cancer with therapeutic strategies including chemotherapy has generally been disappointing due to high systemic toxicity and drug resistance limits the successful outcomes in most cases. It is most desirable to have more effective new drugs (Chi-Kuan *et al*., 2002) [2]. One such approach could be a combination of an effective photochemical with chemotherapeutic agents which when combined would enhance efficacy while reducing toxicity to normal tissues.Increasing attention is being paid to primitive medicinal plants and dietary factors to search for new substances with potentially effective anti-cancer activity.Several studies have been designed to examine the anticancer activities of xanthones isolated from mangosteen-fruit pericarp. Table 3 represents antitumoral properties of xanthones isolated from *Garcinia mangostana*.

Table 3: Antitumoral properties of xanthones isolated from GML (Jose *et al*., 2008) [7]

Effect	References
Garcinone E has a cytotoxic effect on hepatoma cells lines as well as on the gastric and lung cancer cell lines	Ho *et al*. (2002) [2]
Six xanthones from the pericarp of GML showed antiproliferative activity against human leukemia HL60 cells. In addition, a-mangostin induced caspase 3-dependent apoptosis in HL60	Matsumoto *et al*. (2003)
The treatment with dietary a-mangostin inhibits cells proliferation in the colon lesions in rats injected with DMH	Nabandith *et al*. (2004)
Aqueous extract of the fruit rind GML showed antileukemic activity in four cells lines	Chiang *et al*. (2004)
a-Mangostin induced apoptosis in human leukemia cell lines	Matsumoto *et al*. (2004)
Ethanolic and methanolic extracts of GML showed antiproliferative effect on human breast cancer SKBR3 cells	Moongkarndi (2004a,2004b)
The antiproliferative effect of a- and c-mangostins, was associated with apoptosis in human colon cancer DLD-1 cells	Matsumoto *et al*. (2005)
a-Mangostin inhibited DMBA-induced preneoplastic lesions in a mouse mammary organ culture	Jung *et al*. (2006)
Mangostenone C, mangostenone D, demethylcalabaxanthone, b-mangostin, gartanin, garcinone E, a-mangostin, mangostinone, c-mangostin, garcinone D, and garcinone C showed cytotoxic effect on the three human cancer cell lines	Suksamrarn *et al*.(2006)
a-Mangostin showed antitumoral activity against DLD-1 cells	Nakagawa *et al*. (2007)

The aim here was to isolate Garcinone E from the pericarp of GML and study the effect of its cytotoxicity on Sp2/0 cell lines.Sp2/0 cells are mouse-myeloma cell lines from B-lymphocyte. They grow indefinitely and can be used as fusion partners for B cells in the production of hybridomas. They are used in research because they functionally behave as myeloma cells.

Organism: *Mus musculus* (B cell) ;*Mus musculus* (myeloma), mouse (B cell) ; mouse (myeloma)
Strain: BALB/c
Tissue: Spleen
Cell Type: Hybridoma: B lymphocyte
Morphology: Lymphoblastlike
Growth Properties: Suspension

Materials and Methods

Preparation of Pericarp Extract of *Garcinia mangostana* Linn (Zarena *et al*., 2009) [26]

To prepare pericarp extract of Garcinia *mangostana* Linn (mangosteen fruit) using Soxhlet Apparatus. The following materials and methods were used to obtain the extract.

Collection of Fruit Material

Fresh Mangosteen (*Garcinia mangostana* Linn) fruits (2kg) was purchased from Nilgris, Coimbatore and washed with tap water for 2-3 times and finally with distilled water to remove dust particles. The pericarp of the fruit was cut into small pieces and shade dried indoors with good ventilation for 3 days. After drying, the pericarp was crushed by hammer, pulverized well using a grinder and sieved to provide fine powder and stored in an air tight container for future use.

Preparation of fruit extract (Zarena *et al*., 2009) [26]

The fruit extract was prepared using Soxhlet apparatus (Zarena *et al*., 2009) [26] using two different solvents, Ethyl Acetate and 95% Ethanol.

Soxhlet Extraction with Ethyl Acetate Solvent

The finely powdered pericarp of mangosteen fruit (30g) was subjected to extraction with ethyl acetate solvent.

Materials and reagents required

30g of pericarp powder, 300ml of ethyl acetate solvent, absorbent cotton.

Procedure

30g of pericarp powder was weighed and taken in the thimble of the soxhlet apparatus and subjected to extraction with 300ml of ethyl acetate solvent taken in the round bottom flask for 2 hours at a temperature of 75 ºC. The ethyl acetate extract obtained at the end of the extraction process was dried in petriplates, weighed and the yield was calculated. The result was expressed in yield %.

Soxhlet Extraction With Ethanol Solvent

The finely powdered pericarp of mangosteen fruit (30g) was subjected to extraction with 95% ethanol solvent.

Materials and Reagents Required

30g of pericarp powder, 300ml of 95% ethanol solvent, absorbent cotton.

Procedure

30g of pericarp powder was weighed and taken in the thimble of the soxhlet apparatus and subjected to extraction with 300ml of 95% ethanol solvent taken in the round bottom flask for 8-10 hours at a temperature ranging between 60 to 75ºC.The extract obtained was dried in petriplates, weighed and the yield was calculated. The result was expressed as yield %.

Isolation of Garcinone E Using Column Chromatography

To separate the different compounds present in the extract and isolate the xanthone Garcinone E using Column Chromatography for the different solvents, ethyl acetate and 95% ethanol. A silica gel column was prepared and the ethyl acetate extract and the ethanol extract were eluted separately with different solvents.

Ethyl Acetate Extract (Hyun-AH Jung *et.al*., 2006) [6]

The ethyl acetate extract was eluted through the silica gel column. The column was prepared with silica gel and solvents choloroform and methanol in 1:1 ratio.

Materials and Reagents Required

Glass beads, glass wool, silica gel, chloroform: methanol, ethyl acetate extract.

Procedure

The obtained ethyl acetate extract was dissolved in 3ml of 1:1 preparation of chloroform and methanol. This sample was

subjected to chromatography over a silica gel column eluted with a mixture of chloroform and methanol with increasing polarity (ratio of chloroform/methanol from 100:1 to 1:1).
The eluents required for the chromatographic separation were prepared as per Table 4.

Table 4: Preparation of eluents for ethyl acetate extract

Fractions	Eluent ratios Chloroform: Methanol	Chloroform (ml)	Methanol (ml)
F1	100:1	10	0.1
F2	95:1	9.5	0.1
F3	90:1	9	0.1
F4	85:1	8.5	0.1
F5	80:1	8	0.1
F6	75:1	7.5	0.1
F7	70:1	7	0.1
F8	65:1	9.75	0.15
F9	60:1	9	0.15
F10	55:1	8.25	0.15
F11	50:1	7.5	0.15
F12	45:1	10.125	0.225
F13	40:1	9	0.225
F14	35:1	7.875	0.225
F15	30:1	6.75	0.225
F16	25:1	7.5	0.3
F17	20:1	6	0.3
F18	15:1	9	0.6
F19	10:1	6	0.6
F20	5:1	8	1.6
F21	1:1	4	4

5ml of the prepared eluents was loaded onto the column to carry out chromatographic separation. The fractions were collected in 21 different test tubes. These fractions were then subjected to UV spectrophotometric analysis.

Ethanol Extract (Jun-Jie Koh *et al*., 2013)
The ethanol extract was eluted through a silica gel column. The column was prepared with silica gel and solvents petroleum ether and acetone in 1:1 ratio.

Materials and Reagents Required
Glass beads, glass wool, silica gel, petroleum ether : acetone, ethanol extract.

Procedure
The obtained ethanol extract was partitioned with of ethyl acetate to obtain an ethyl acetate soluble fraction. 3ml of this fraction was subjected to chromatography over a silica gel column eluted with a mixture of petroleum ether and acetone with increasing polarity (ratio of chloroform/methanol from 100:1 to 1:1)**.** The eluents required for the chromatographic separation were prepared as per Table 5.

Table 5: Preparation of eluents for ethanol extract

Fractions	Eluent ratios Petroleum Ether : Acetone	Petroleum Ether (ml)	Acetone (ml)
F1	10:1	10	1
F2	9:1	9	1
F3	8:1	8	1
F4	7:1	7	1
F5	6:1	6	1
F6	5:1	10	2
F7	4:1	8	2
F8	3:1	6	2
F9	2:1	8	4
F10	1:1	4	4

5ml of the prepared eluents was loaded onto the column to carry out chromatographic separation. The fractions were collected in 10 different test tubes. These fractions were then subjected to UV spectrophotometric analysis.

Characterization of Xanthones
Uv Spectrophotometric Analysis (Werayut *et al*., 2008) [23]
This method is used for quantification of total mangostins content in the extracts obtained from the hulls of *Garcinia mangostana* Linn. The fractions obtained from both ethyl acetate extract and ethanol extract were subjected to UV analysis from the wavelength ranging from 190-400nm. The results were expressed in nm units.

Hplc Characterization of Garcinone E (Zarena *et al*, 2009) [26]
The isolated Garcinone E from ethanolic extract was subjected to further characterization by HPLC.The result was expressed as retention time in mins.

Materials and Reagents Required
0.1% (v/v) acetic acid in water (ELUENT A), 95% (v/v) methanol (ELUENT B), HPLC grade water.

Procedure
- The column used was RP-C18, 150X4.6mm,SS, Exsil ODS 5µM particle size operated at a temperature of 40 ºC.
- The mobile phase consisted of 0.1% (v/v) acetic acid in water- ELUENT A and 95% (v/v) methanol – ELUENT B.
- The gradient program was as follows:
- 65 – 90% B over 0-40 mins at the flow rate of 1.0ml/min.
- 20µl of the ethanolic extract sample was introduced through the rotary injection valve while in the LOAD position and UV detection was recorded at 254nm.

Culturing of Sp2/0 Cell Lines (Yong-zhan *et al*., 2009) [24]
SP2/0 cell lines was obtained from Bioklone, Chennai and stored in liquid nitrogen to ensure best quality.

Materials and Reagents
IMDM medium, 10% Fetal bovine serum (FBS), 1% antibiotic (penstrep), Suspension T- Flask, syringe filters, serological pipettes, centrifuge tubes.

Procedure
The cancer cell line was cultured in IMDM medium solution with 10% Fetal Bovine Serum (FBS) and 0.1% antibiotic in a humidified incubator with an atmosphere of 5% CO2 in air at 37ºC. The cells were grown as a suspension culture in T flasksand then the cell density was calculated using Tryphan blue method. The results were expressed as % viability of Sp2/0 cells.

Treating the Sp2/0 Cells With Garcinone E (Yong-zhan *et al*., 2009) [24]
The cultured Sp2/0 cells were treated with varying volumes of the isolated garcinone E.

Materials Required
96 well plate, 1 million Sp2/0 cells/ml, Garcinone E concentrations, pipettes.

Procedure
- The cell suspension containing approx 1 million cells/ml

was seeded onto a 96 well plate.
- For this, 100µl of the cell suspension was taken in each well.
- The cell density of each well was therefore a constant containing 10,000 cells.
- To these wells different concentrations of garcinone E from 10µl, 50 µl, 100µl, 200µl, 250 µl and 500µl was added and incubated for 6, 12, 24, 48 hours.
- This was performed in triplicates for each concentration along with a control.

***In vitro* Cytotoxicity Test**

Cytotoxicity test was done to check the killing activity of the drug against the cancer cells. This was performed using Tryphan blue dye exclusion method and MTT assay.

Tryphan Blue Dye Exclusion Method (Sanjay *et al*., 2009)

Principle

Tryphan Blue is a blue acid dye that has two azo chromophores group.It is an essential dye used in estimating the number of viable cells present in cell suspension.It is based on the principle that live cells possess intact cell membrane that excludes certain dyes such as tryphan blue, eosin or propidium, whereas dead cells do not.

Materials and reagents required

0.4% Tryphan blue dye, Hemocytometer chamber, coverslip.

Procedure

- 10µl of tryphan blue was mixed with 10µl of cell suspension.
- It was then loaded carefully into a hemocytometer chamber and viewed under the microscope.
- The number of live cells and dead cells were counted and the cell viability was calculated for different concentrations using the formula :

%viability = (no. of live cells / total no. of cells) × 100

The results obtained after calculations were expressed in % viability of the Sp2/0 cells.

Mtt (Microculture Tetrazolium) Assay (Mosmann *et al*., 1983) [12]

Principle

This colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4,5-dimethyl triazol-2-yl)-2, 5-diphenyl tetrazolium bromide into an insoluble formazan product which is measured spectrophotometrically.Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of viability of cells.

Materials and Reagents Required

- MTT dye solution: 5mg of MTT dye powder was mixed with 1ml of phosphate buffered saline (PBS).
- DMSO(Dimethyl sulphoxide)

Procedure

- 10µl of MTT dye was added to the well plates which were incubated for 24 hrs and 48 hrs, including the control.
- It was washed gently with PBS.
- The plates were gently shaken and incubated for 3hrs at 37 ºC in 5% CO_2incubator.
- After the incubation, 100µl of DMSO (stop solution) was added to the well plates to solubilize the formed formazan.
- The plate was observed under the microscope to check for crystal formation.
- The absorbance was measured at 630nm using ELISA plate reader.
- The percentage viability was calculated using the following formula:

%cell viability = {(At-Ab) /(Ac-Ab)} x 100

where,
At = Absorbance value of test compound
Ab = Absorbance value of blank
Ac = Absorbance value of control

The results obtained were expressed in % viability of the Sp2/0 cells.

Results

2 kg of fresh mangosteen fruit purchased from Nilgiris – Coimbatore had 32 fruits. For the 32 fruits that were taken, the total weight of dried pericarp was found to be 650g. A yield of 310g of fine pericarp powder was obtained. This powder was stored in an air tight container for future use.
Fig 3 represents the fine pericarp powder obtained after grinding the pericarp.

Fig 3: Fine pericarp powder of G. mangostana

Preparation of fruit extract

For ethyl acetate, the total weight of dried extract obtained was 3.71g for 30g of pericarp powder used. The yield was calculated to be 12.36%.

Fig 4: Ethyl acetate extract after soxhlet extraction

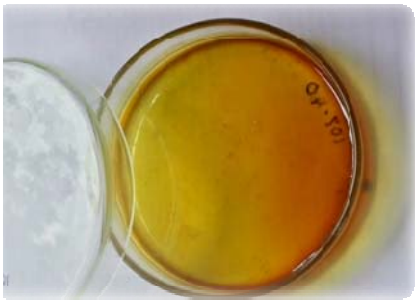


Fig 5: Dried ethyl acetate extract in petriplate

For ethanol, the total weight of dried extract obtained was 9.58g for 30g of pericarp powder used. The yield was calculated to be 31.9%.

Fig 6: Ethanol extract obtained after soxhlet extraction

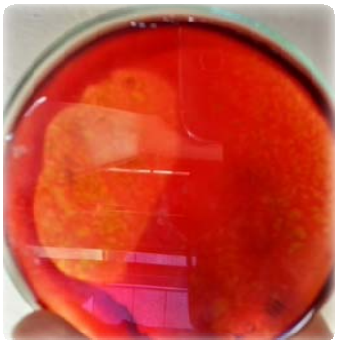


Fig 7: Dried ethanolic extract in petriplate

Fig 8 shows the dried extract resuspended in water and partitioned with ethyl acetate to obtain an ethyl acetate soluble fraction which was used for the isolation of Garcinone E.

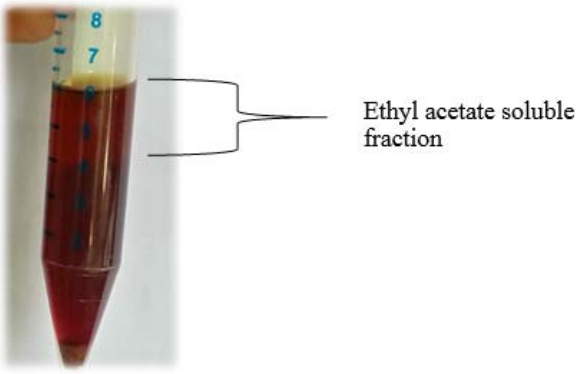


Fig 8: Ethanol extract partitioned with ethyl acetate

Isolation of Garcinone E using Column Chromatography

21 fractions were collected for the ethyl acetate extract and 10 fractions were obtained for the ethanolic extract. These were collected in separate test tubes and then subjected to UV spectrophotometric analysis.

Uv Spectrophotometric Analysis (Werayut *et.al*., 2008) [23]

The results for UV analysis of the 21 fractions eluted is given in Table 6 and the results for UV analysis of the 10 fractions eluted is given in Table 7.

Table 6: UV Results for ethyl acetate extract

Fractions	Polarity	Peaks observed
F1	100:1	216,236,238
F2	95:1	236,242,284,326
F3	90:1	218,248,268,272,278,350
F4	85:1	234,284,396
F5	80:1	236,248
F6	75:1	216,276
F7	70:1	214,248,322,342
F8	65:1	232, 280, 348
F9	60:1	216, 236, 384
F10	55:1	304, 370
F11	50:1	238
F12	45:1	216, 234, 356
F13	40:1	236, 264, 358
F14	35:1	236,358
F15	30:1	358
F16	25:1	358
F17	20:1	224, 358
F18	15:1	214
F19	10:1	224, 314, 358
F20	5:1	236, 314
F21	1:1	236, 264, 314

F1- F21 are fractions in volume (ml)
Polarity is expressed in ratio of Chloroform:Methanol

Table 7: UV Results for ethanol extract

Fractions	Polarity	Peaks observed (nm)
F1	10:1	307, 287, 253, 212
F2	9:1	215, 235, 275, 307
F3	8:1	215, 238, 247, 285, 312
F4	7:1	216, 239, 250, 265, 293,310,325
F5	6:1	213, 238, 247, 253, 270, 317, 341
F6	5:1	208, 249, 270, 306, 353
F7	4:1	213, 239, 246, 250, 271, 312, 323
F8	3:1	210, 235, 279, 349
F9	2:1	213, 235, 253, 285
F10	1:1	212, 225, 236, 253, 285

F1- F10 are fractions in volume (ml)

Polarity is expressed in ratio of Petroleum Ether:Acetone

UV spectrophotometer analysis revealed that the Fraction 5(F5) with polarity 6:1 obtained from the silica gel column using ethanolic extract and eluents Petroleum ether/acetone showed peak at 253nm which corresponded to the standard peak of garcinone E recorded at 254nm (Zarena *et.al*., 2009) [26]. Hence further studies were done with this fraction. Fig 9 shows the UV spectrum for fraction 5 of ethanol extract.

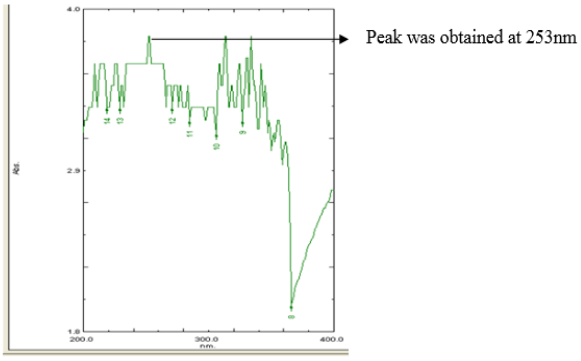


Fig 9: UV Spectrum for fraction 5 of ethanol extract

Characterization Of Garcinone E Using Hplc Analysis
Fig 10 shows the HPLC Peak for Garcinone E standard.
Fig 11 shows the HPLC peak for xanthones present in Fraction 5 of ethanol extract.

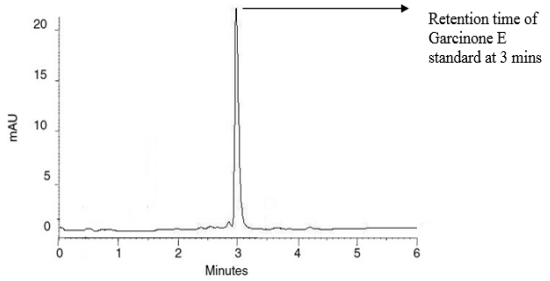


Fig 10: HPLC Peak for Garcinone E standard

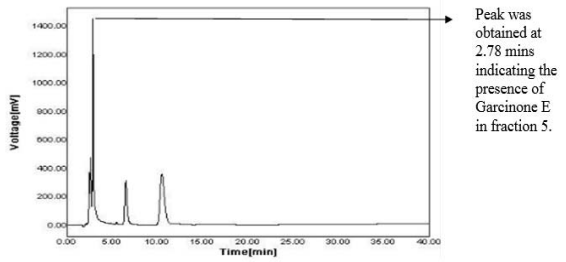


Fig 11: HPLC peak for xanthones present in Fraction 5 of ethanol extract

The peak was recorded for the sample at 254nm and compared with the peak available for the standard. On comparison, it was found that the retention time of Garcinine E was 2.78 mins.

Culturing Of Sp2/0 Cell Lines
The cell density of the cultured Sp2/0 cells was calculated and found to be ~6million cells/ml.
Live cells = 337+320+416 +272 = 1345/4 = 336
Dead cells = 85+75+67+72 = 299/4 = 74.75

The cell density was calculated using the following formula:
Cell Density: no of live cells × dilution factor × 10^4 cells/ ml
= 336 × 2 × 10^4
= 67,20,000 cells/ml

This was then diluted with 6ml of medium to obtain a cell density of 1million cells per ml.
Fig 22 depicts how the cultured Sp2/0 cells appeared like when viewed under the fluorescent microscope.

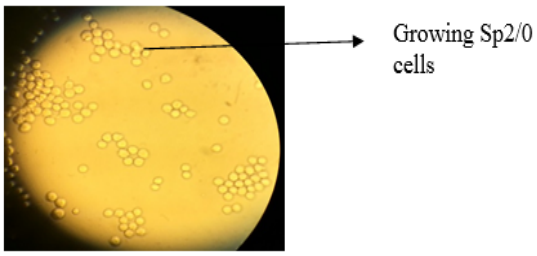


Fig 12: Microscopic view of live Sp2/0 cells

Treating The Sp2/0 Cells With Garcinone E
Tryphan Blue Dye Exclusion Method
Fig 13 shows the microscopic view of the live and dead cells after the incubation of the live Sp2/0 cells with Garcinone E.

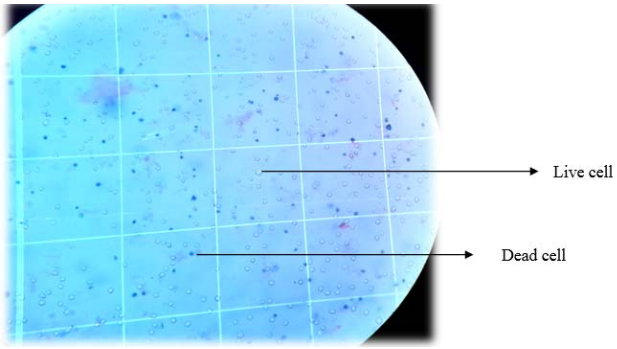


Fig 13: Microscopic view of the hemocytometer showing live and dead cells

The tryphan blue results for various incubation times of different volumes of Garcinone E with the Sp2/0 cells are given in the following tables, Table 8 to Table 11.
Live and dead cells were expressed in numbers
% Viability was calculated and expressed in percentage

Table 8: 6 hours incubation

Volume of Garcinone E (µl)	Live cells (In number)	Dead cells (In number)	% Viability
10	9210±10	790±10	92.10±0.01
50	8860.33±4.33	1139.67±4.67	88.60±0.04
100	8295±5	1705±2	82.95±0.05
200	7901±6	2099±5	79.01±0.06
250	6699.33±6.33	3300.67±5.67	66.99±0.06
500	6263.33±6.33	3736.67±3.33	62.63±0.03

Table 9: 12 hours incubation

Volume of Garcinone E (µl)	Live cells	Dead cells	% Viability
10	8826.66±6.67	1173.33±6.67	88.26±0.06
50	7436.66±3.33	2563.33±3.33	74.3±0.03
100	6876±5	3123±5	68.76±0.05
200	6210±10	3790±10	62.10±0.10
250	5246.67±3.33	4753.33±3.33	52.46±0.03
500	5713.33±13.33	4286.67±13.33	57.13±0.13

Table 10: 24 hours incubation

Volume of Garcinone E (µl)	Live cells	Dead cells	% Viability
10	7295±5	2705±5	72.95±0.05
50	6193.33±6.67	3806.67±6.67	61.93±0.07
100	5868±8	4132±8	58.68±0.08
200	5453.67±6.33	4546.33±6.33	54.53±0.07
250	4806.67±6.67	5193.33±6.67	48.07±0.07
500	5111.67±8.33	4888.33±8.33	51.12±0.08

Table 11: 48 hours incubation

Volume of Garcinone E (µl)	Live cells	Dead cells	% Viability
10	6255.67±4.33	3745±5	62.55±0.05
50	4964±14	5036±14	49.64±0.14
100	4702.67±4.67	5297.33±7.33	47.02±0.03
200	4405.33±5.33	5594.67±6.33	44.05±0.05
250	3810.67±9.33	6172.67±7.33	38.11±0.09
500	3805.67±5.67	6194.33±5.67	38.06±0.06

The graphical representation of the data in the above tables is given in Fig. 14 whichrepresents the % viability of Sp2/0 cells after 6, 12, 24 and 48 hours incubation with Garcinone E.

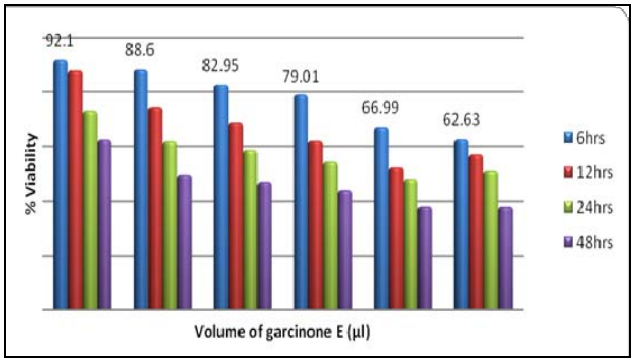


Fig 14: Viability of Sp2/0 cells post incubation with Garcinone E

Mtt (Microculture Tetrazolium) Assay

The O.D values along with % viability calculated according to the mentioned formula is given in Tables 12 to 15.

Table 12: 6 hrs incubation

Volume(µl)	Well 1	Well 2	Well 3	Avg	% Viability
10	0.205	0.210	0.221	0.212	92.80
50	0.196	0.198	0.195	0.196	84.07
100	0.187	0.190	0.188	0.188	79.62
200	0.184	0.176	0.180	0.180	75.00
250	0.160	0.176	0.167	0.168	68.30
500	0.156	0.142	0.138	0.154	60.55

Table 13: 12 hrs incubation

Volume(µl)	Well 1	Well 2	Well 3	Avg	% Viability
10	0.208	0.195	0.190	0.197	84.44
50	0.185	0.176	0.173	0.178	73.90
100	0.170	0.167	0.161	0.165	66.71
200	0.150	0.156	0.164	0.156	62.20
250	0.148	0.142	0.146	0.145	55.74
500	0.144	0.141	0.139	0.141	53.51

Table 14: 24 hrs incubation

Volume(µl)	Well 1	Well 2	Well 3	Avg	% Viability
10	0.209	0.170	0.203	0.194	82.77
50	0.186	0.161	0.181	0.176	72.78
100	0.167	0.141	0.183	0.164	66.11
200	0.150	0.132	0.124	0.137	50.18
250	0.126	0.108	0.114	0.116	39.44
500	0.113	0.109	0.124	0.115	39.07

Table 15: 48 hrs incubation

Volume(µl)	Well 1	Well 2	Well 3	Avg	% Viability
10	0.187	0.176	0.183	0.182	76.12
50	0.162	0.157	0.152	0.157	62.27
100	0.148	0.141	0.139	0.142	53.90
200	0.132	0.128	0.126	0.128	46.14
250	0.120	0.109	0.115	0.114	38.52
500	0.104	0.112	0.115	0.110	36.24

Avg Absorbance of Control = 0.225
Avg Absorbance of Blank = 0.045

The comparision of the effectiveness of Garcinone E at the different volumes and incubation times with the Sp2/0 cells is given in Fig 15.

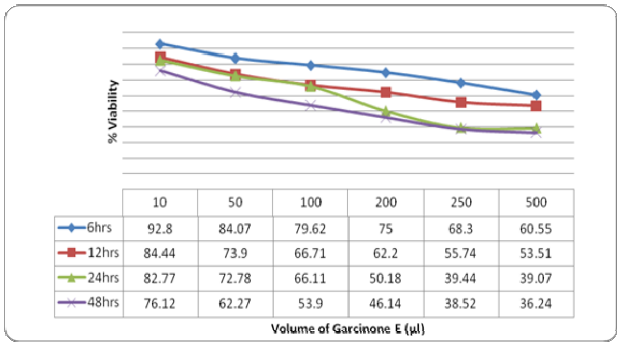


Fig 15: Effectiveness of Garcinone E at different volumes and incubation times

Discussion

A lot of emphasis is being paid at identifying new and effective therapeutic products to serve as a substitute for the substances that are currently being used in various industries. To reduce the incidence of degenerative diseases including cancer, heart diseases and inflammation, research work is being focussed on identifying new and effective phytochemicals with chemotherapeutic properties which would enhance efficacy while reducing toxicity to normal tissues. For this purpose, increasing attention is being paid to primitive medicinal plants and dietary factors to search for new substances with potentially effective therapeutic property. A large number of natural products have been evaluated as potential chemo preventive or therapeutic agents (Yukihiro *et al.*, 2008) [25].

The survey of literature reveals that many tropical plants have biological activities with potential therapeutic applications. *Garcinia mangostana* Linn is a tropical fruit available in Southeast Asia like Indonesia, Malaysia, Sri Lanka, Philippines and Thailand. People have used the pericarp (peel, hull, rind or ripe) of GML as a traditional medicine for the treatment of abdominal pain, infected wounds, inflammation, skin infections, suppuration, and chronic ulcer (Jose *et al.*,2008) [7]. Out of the 50 xanthones that have been isolated from the pericarp of the mangosteen fruit, α-mangostin, β-mangostin, γ-mangostin, Garcinone E, Gartanin and 8-

deoxygartanin are some of the most studied xanthones (Sen *et al*., 1980) [17]. Ho *et al*., (2002) [2] evaluated that Garcinone E has potent cytotoxic effect against hepatocellular carcinoma cell lines.

In the present study, Garcinone E is isolated from the pericarp of the mangosteen fruits using soxhlet apparatus. Two solvents ethyl acetate and 95% ethanol were used to obtain pericarp extracts. On comparing both extracts, it was found that the ethanolic extract gave more yield ~30% when compared to 12.35% yield of ethyl acetate extract obtained. This could be due to the higher polarity and other physical properties of ethanol. This similar study was conducted by (Hyun-Ah Jung *et al*., 2006) [6]. Garcinone E was isolated from the crude ethanolic extract using column chromatography over a silica gel column (Jun-Jie Koh *et al*., 2013). It was then characterized using UV spec and HPLC which revealed that fraction 5 collected from chromatographic elution of the ethanolic extract showed a peak corresponding to the standard garcinone E peak at 253nm. Further research was therefore carried out using the characterized Garcinone E which showed a retention time of 2.78mins during HPLC analysis (Zarena *et al*., 2009) [26] The cytotoxic effect of garcinone E on Sp2/0 cell lines was studied using tryphan blue dye exclusion method and MTT assay. Sp2/0 is a non-Ig-secreting or synthesising line derived from a cell line created by fusing a BALB/c mouse spleen cell and the mouse myeloma P3X63Ag8. Functionally it behaves as a myeloma cell line (Yong-zhan *et al*., 2008).

Different volumes of garcinone E such as 10µl, 50µl, 100µl, 200µl, 250µl, and 500µl was treated with Sp2/0 cells taken in a 96 well plate containing 10,000 cells per well. The time period of incubation was varied between 6, 12, 24, and 48 hours. On comparing the results obtained from Tryphan blue dye exclusion method and MTT assay, it was found that Garcinone E had cytotoxic effect against Sp2/0 cells. The optimumvolume of Garcinone E required to kill the maximum number of Sp2/0 cells was found to be 250µl with optimum incubation time of 48 hrs. This was concluded because despite increasing the volume of garcinone E to 500µl, the number of dead cells observed did not vary considerably with an increase in the volume.

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