

## 1 ORIGINAL ARTICLE

2 **Studies of Cytotoxic Potential of *Cucumis melo*. Linn**  
3 **Fruit Aqueous Extract in Prostate Cancer Cell lines**  
4 **PC-3 Using MTT and Neutral Red Assay**5 SIBI P ITTIYAVIRAH, ANN GEORGE, ANJU M SANTHOSH, SUDHI T KURIAN,  
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8 Received May 12, 2012; Revised October 29, 2012; Accepted December 8, 2012

9 This paper is available online at <http://ijpt.tums.ac.ir>10 **ABSTRACT**11 The objective of the study was to evaluate the cytotoxic effects of aqueous fruit extract of *Cucumis melo*  
12 in human prostate cancer cell line (PC-3) using MTT and neutral red assays. The crude aqueous extract  
13 of *C. melo* was prepared by cold maceration method, filtered, concentrated and tested on PC-3 cell line.  
14 Dose-dependent cytotoxic activities were exhibited by human prostate carcinoma PC-3 cell line. As the  
15 dose of the extract increased, the number of viable cells decreased. This confirms the anti-cancer and  
16 cytotoxic potential of the fruit of *C. melo*.17 **Keywords:** Cytotoxicity, MTT assay, Neutral red assay, Human prostate carcinoma cell lines (PC-3),  
18 *Cucumis melo*19 Carcinoma of the prostate gland is the malignancy of 41 carbohydrates, Proteins, fibre, citric acid, vitamin K,  
20 the male genitourinary tract and is a disorder in older 42 vitamin A and folate. Traditionally, it is used for  
21 men, with mean age at presentation about 70 years 43 treatment of Kidney stones, cancer, cardiovascular  
22 when diagnosed by the presence of symptoms. The risk 44 disorders and stroke. Three components found in  
23 of developing prostate cancer is affected by racial and 45 melons are Cucurbitacin- $\beta$ , Lithium and Zinc which  
24 environmental factors. The magnitude of familial risk 46 exhibit promise in cancer prevention, fighting  
25 increases with number of first degree relatives who are 47 depression, dandruff, and ulcers and stimulates the  
26 affected, and also if the affected relatives diagnosed 48 immune system. *C. melo* is also rich in antioxidants,  
27 with prostate cancer at an early age. Prostate cancer risk 49 flavonoids such as  $\beta$ - carotene, lentin, xanthin and  
28 has been inversely associated with several dietary 50 cryptoxanthin. These antioxidants have the ability to  
29 components including the essential non-metallic trace 51 protect cells and structures in the body from oxygen free  
30 element selenium. Chromosomal alterations are 52 radicals, hence offer protection against prostate, colon,  
31 associated with an inherited predisposition to prostate 53 breast, lungs, endometrial and pancreatic cancer. The  
32 cancer and prostate cancer and its development. Two 54 cucurbitacea family includes several species of  
33 prostate cancer susceptibility genes that have been 55 cultivated plants that has great economic importance  
34 identified are the RNASEL and MSR1 genes both of 56 like water melon (*Citrullus lanatus*.L), squash  
35 which are associated with response to infections. 57 (*Cucurbita maxima*.L), cucumber (*Cucumis sativus*.L)36 *Cucumis melo* fruit is round in shape, tan to greenish 58 and cantaloupe(*Cucumis melo*.L) [1]. Earlier studies on  
37 tan with a rough texture and orange pink flesh. It is well 59 cucurbitacea family showed that cantaloupe pulp extract  
38 known for its sweet taste and fragrance. It is native to 60 possesses high antioxidant and anti-inflammatory  
39 Persia, Armenia, etc. Many phytochemicals having 61 properties [2]. The active principles in the vegetable  
40 potential benefits are present in *C. melo*. It is rich in 62 extracts are principally water soluble or lipophilic

antioxidant molecule. Most of these plant extracts was mixed with strained liquid, filtered to make a clear liquid and concentrated.

triterpenoids and other flavanoids [3]. For this, these were used as potential antioxidant prophylactic agents.

for both health and diseases management [3,4]. The methanolic seed extract (MECM) of *Cucumis melo*. Var possess significant antioxidant, anti-inflammatory and analgesic properties [5], while the fruit extract *C. melo* fruit exhibited immunomodulatory activity [6]. Even though a large number of compounds were screened for cytotoxicity and anticancer studies, hardly a few lead compounds had shown promising results. Hence, it was thought to identify potential compounds from our traditional ethno-medicinal knowledge for treatment of kidney, urinary and prostate cancer. In the present study, an initial attempt has made for to scientifically evaluate its anticancer effects. The main aim of the study is to evaluate the cytotoxic effects of aqueous fruit extract of *C. melo* in human prostate cancer cell line (PC-3) using MTT and neutral red assays.

**Alkaloids-Dragendroff's test**

Eight gram of bismuth nitrate was dissolved in 20 ml nitric acid and 2.72 g of potassium iodide in 50 ml water. These were mixed and allowed to stand. When potassium nitrate crystals out, the supernatant was discarded off and made up to 100 ml with distilled water. The alkaloids were regenerated from the precipitate by treating with sodium carbonate followed by extraction of the liberated base with ether. To 0.5ml of alcoholic solution of extract was added 2.0 ml of hydrochloric acid. To this acidic medium, 1.0 ml of reagent was added. An orange red precipitate was produced immediately indicated the presence of alkaloids.

## MATERIALS AND METHODS

### Plant material

*C. melo* fruits were collected from local fruit stall Cherthala, Alappuzha District in the month of November 2012 and authenticated at Department of Environment Sciences, Mahatma Gandhi University Kottayam, Kerala, India. *In vitro* methods were used for assessing the cytotoxic activity and they were in accordance with the guidelines of Institutional Animal Ethical Committee (IAEC).

### Reagents for phyto-chemical analysis

Bismuth nitrate, Nitric acid, Potassium iodide, Sodium carbonate, Mercuric chloride, Sulphuric acid, Hydrochloric acid, Sodium hydroxide, Ferric chloride, Alpha naphthol, Copper sulphate, Zinc chloride 3-(4,5-dimethyl thiazole-2-yl)-2,5-diphenyl tetrazolium bromide(MTT), Isopropanol, Phosphate buffer saline(PBS), Dimethyl sulfoxide (DMSO), Calorimeter, 1M Potassium dihydrogen phosphate, CO<sub>2</sub> incubator, PBS, Elution medium (ethanol/acetic acid), Spectrophotometer.

### MTT assay and neutral red assay

PC-3 cell line-PC-3 prostate cell lines purchased from National centre for Cell Sciences (NCCS), Pune, Maharashtra, India. 1M Potassium dihydrogen phosphate, CO<sub>2</sub> Incubator, PBS, Elution medium (ethanol/acetic acid).

### Preparation of extract: cold maceration

The fruit was washed and the outer skin was peeled off. The remaining fleshy part was cut in to small pieces. Then it was soaked in water for seven days and was kept in a dark place. During this period shaking was done occasionally. After seven days, the liquid was strained and marc was pressed. The expressed liquid

### Mayer's test

The amount of 1.36 g mercuric chloride was dissolved in 60 ml of distilled water and 5 g of potassium iodide in 10 ml of water. The two solutions were mixed and diluted to 100 ml with distilled water. To 1 ml of acidic aqueous solution of extracts, a few drops of reagent was added. Formation of white or pale precipitate showed the presence of alkaloids.

### Wagner's test

The amount of 1.2 g Iodide and 2.0 g of H<sub>2</sub>SO<sub>4</sub> and the solution was diluted to 100 ml. Ten ml of alcoholic extract was identified by adding 1.5% v/v of HCl and a few drops of wagner's reagent. Formation of yellow or brown precipitate confirmed the presence of alkaloid.

### Flavonoids

In a test tube containing 0.5 ml of alcoholic extract, 5-10 drops of dilute HCl and a small piece of ZnCl<sub>2</sub> or Mg were added and the solution was boiled for few minutes. In the presence of flavonoids, reddish pink or dirty brown color was produced.

### Phytosterols

To 2 ml of chloroform extract, 1ml of concentrated sulphuric acid was added carefully along the sides of the test tube. In the presence of phytosterols, a golden yellow color was produced in the chloroform layer.

### Glycosides

A small amount of alcoholic extract was dissolved in 1 ml of water and the aqueous NaOH solution was added. Formation of yellow colour indicated the presence of glycosides.

**Table 1.** Phytochemical constituents aqueous extract of *C. melo*

Tests	Observation	Inference
<b>Alkaloids</b>		
Dragendroff's test	Orange red ppt was observed	Presence of alkaloids (+)
Mayer's test	Pale white ppt was observed	Presence of alkaloids (+)
<b>Flavanoids</b>	Dirty brown ppt was observed	Presence of Flavanoids (+)
<b>Phytosterols</b>	Dark golden colour was observed	Presence of phytosterols (+)
<b>Glycosides</b>	No characteristic change was observe	Absence of glycosides (-)
<i>Tannins</i>	No characteristic change was observed	Absence of glycosides (-)
<b>Carbohydrates</b>		
Molisch's test	No characteristic change was observed	Absence of carbohydrates (-)
<b>Proteins</b>		
Biurett's test	No characteristic change was observed	Absence of proteins (-)
<b>Saponins</b>	No characteristic change was observed	Absence of saponins (-)

**Table 2.** Cytotoxicity studies aqueous extract of *C. melo* using MTT

Sample concentration (µg/ml)	OD (540 nm)	% viability
Control	0.220	100
100	0.211	95.90
500	0.172	78.18
1000	0.148	67.27

**167 Tannins-Ferric chloride test**

168 To 1-2 ml of aqueous extract, few drops of 5%  
169 aqueous ferric chloride solution was added. A bluish  
170 black color which disappears on addition of a few ml of  
171 sulphuric acid, there is no formation of yellowish brown  
172 precipitate.

**173 Sugars-Molish's test**

174 In a test tube containing 2 ml of aqueous extract, 2  
175 drops of freshly-prepared 20% alcoholic solution of α-  
176 naphthol was added and mixed. To this solution, 2 ml of  
177 conc: Sulfuric acid was added so as to form a layer  
178 below the mixture. Formation of red violet ring at the  
179 junction of solution and its disappearance on the  
180 addition of an excess solution indicated the presence of  
181 carbohydrates.

**182 Proteins-Biurett's test**

183 In a test tube containing 2 ml of test sample, 2 ml of  
184 10% NaOH is added and mixed well. Then 0.1% CuSO<sub>4</sub>  
185 solution is added. A violet or pink colour indicated the  
186 presence of proteins.

**187 Saponins-Froth Test**

188 Few ml of the extract is transferred in to a test tube  
189 and shaken vigorously then is left to stand for 10 min. A  
190 thick persistent froth indicated presence of saponins.

**191 Cytotoxicity studies [7]****192 MTT assay**

193 MTT is a colorimetric assay that measures the  
194 reduction of yellow 3-(4, 5-dimethylthiazol-2-yl)-2, 5-

195 diphenyl tetrazolium bromide (MTT) by mitochondrial  
196 succinate dehydrogenase. The MTT enters the cells and  
197 passes into the mitochondria where it is reduced to an  
198 insoluble, colored (dark purple) formazan product. The  
199 products are then solubilized with an organic solvent  
200 (eg. isopropanol) and the released, solubilized formazan  
201 reagent. Since reduction of MTT can only occur in  
202 metabolically-active cells, the level of activity is a  
203 measure of the viability of the cells

204 PC-3 human prostate cell lines purchased from  
205 NCCS Pune, was maintained in Dulbecco's Modified  
206 Eagles Media (DMEM) and grown to confluency at  
207 37°C and 5% CO<sub>2</sub> in a humidified atmosphere in a CO<sub>2</sub>  
208 incubator. The cells were trypsinized (500 µl of 0.025%  
209 Trypsin in PBS/ EDTA solution) for 2 min and  
210 passaged to T flasks in complete aseptic conditions and  
211 incubated. Extracts were added to 80% confluent cells  
212 at a concentration of 100 µg, 500 µg and 1000 µg from a  
213 stock of 100 mg/ml and incubated for 24 h. The cell  
214 culture suspension was washed with 1× PBS. Then, 200  
215 µl MTT solutions were added to the culture (MTT: 5  
216 mg/volume dissolved in PBS). These were incubated at  
217 37°C for 3 hours. All MTT wash was removed with 1×  
218 PBS and 300 µl DMSO was added to each culture. The  
219 plates were incubated at room temperature for 30 min  
220 until the cell get lysed and color was obtained. The  
221 solution was transferred to centrifuge tubes and  
222 centrifuged at top speed for 2 min to precipitate cell  
223 debris. Optical density (OD) was read at 540 nm using  
224 DMSO as blank.

**225 Neutral red assay**

226 The neutral red cytotoxicity test was based on the  
227 ability of living cells to uptake and bind neutral red  
228 (NR). NR was a positively-charged dye that easily  
229 diffuses through the cellular membrane of the cells,  
230 accumulates in the cellular cytoplasm and stores in the  
231 acidic environment of lysosomes. The principle of the  
232 test consists in the fact that NR are able to absorb and  
233 bind only with live cells while this ability declines in  
234 damaged or dead cells. The amount of accumulated NR  
235 was thus directly proportional to the amount of live cells  
236 in the cell culture. The pH of the neutral red solution

**Table 3.** Cytotoxicity studies aqueous extract of *C.melo* using Neutral red assay

Sample concentration ( $\mu\text{g/ml}$ )	OD (540 nm)	% viability
Control	0.086	100
100	0.070	81.39
500	0.065	75.58
1000	0.057	66.27

237 was adjusted in all the experiments to 6.35 with the  
238 addition of potassium dihydrogen phosphate (1M), 10  $\mu\text{l}$   
239 of neutral red solution was added to plates and  
240 incubated for 3 h in  $\text{CO}_2$  incubator at 37°C. Cells were  
241 then washed with phosphate buffer saline (PBS) and  
242 fixed with 200  $\mu\text{l}$  of fixing solution. One ml of the  
243 elution medium (ethanol/ acetic acid, 50%/1%) was  
244 added followed by gentle shaking for 10 min, so that  
245 complete dissolution was achieved. Aliquots of the  
246 resulting solutions were transferred to cuvettes and the  
247 absorbance at 540 nm was recorded using the  
248 spectrophotometer.

249

## RESULTS

### 250 Phytochemical analysis

251 The medicinal value of these plants lies in some  
252 chemical active substances that produce a definite  
253 physiological action on the human body. The most  
254 important of these bioactive constituents of plants are  
255 alkaloids, tannins, flavonoids and phenolic compounds  
256 [8]. The Phytochemical screening of the aqueous  
257 extracts of plant sample revealed the presence of  
258 alkaloids and flavonoids and phytosterols (Table 1) [9].

### 259 Cytotoxic studies

260 MTT results showed that 1000  $\mu\text{g/ml}$  aqueous  
261 extract of *Cucumis melo* showed 67.27% (Table 2, Fig  
262 1) while the neutral red uptake assay showed 66.27%  
263 viability (Table 3, Fig 2). The photograph of PC-3 cell

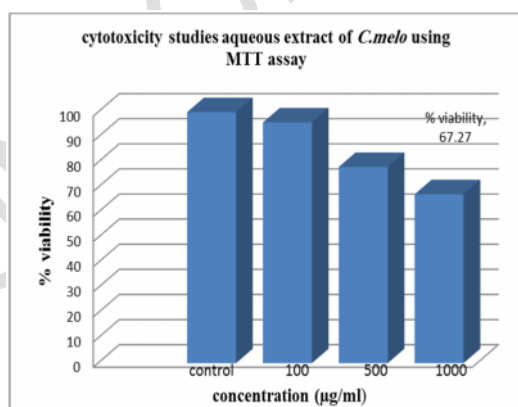
264 line is taken using inverted illuminating microscope  
265 while the control received vehicle and treatment groups  
266 were received *C.melo* at a conc.100 $\mu\text{g/ml}$  and 1000  
267  $\mu\text{g/ml}$  (Fig 3). The results indicate that the crude  
268 aqueous extract of *C. melo* on PC-3 cell lines had shown  
269 a dose-dependent anti proliferative effect. The  $\text{IC}_{50}$   
270 values of MTT and Neutral red assays were found to be  
271 1470 and 1860  $\mu\text{g/ml}$  respectively (Figs 4 and 5).

272

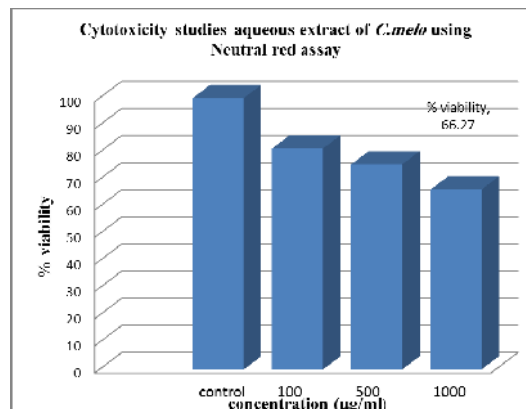
## DISCUSSION

273 Metastatic prostate carcinoma is associated with a  
274 high morbidity and mortality rate with a medium  
275 survival of approximately, 12–15 months. Available  
276 treatment alternatives include radiotherapy after radical  
277 retropubic prostatectomy, radical prostatectomy, and  
278 external beam radiation, prostate brachy therapy, and  
279 androgen ablation of the prostate. Until recently, despite  
280 androgen suppression, no cytotoxic agent has been able  
281 to change the progression of metastatic prostate cancer.  
282 Androgen ablation therapy remains the main course of  
283 treatment with advanced disease. However, it has no  
284 effect on hormone-independent cancer cells.  
285 Chemotherapeutic agents result in less than a 10%  
286 response in advanced prostate carcinoma, in part due to  
287 increased resistance of androgen-independent cells to  
288 apoptosis. However, the severe side effects of  
289 chemotherapy have remained a major problem.

290 In recent years considerable efforts have been made  
291 to identify naturally-occurring compounds and related  
292 synthetic agents can prevent the development and  
293 recurrence of cancer. A wide variety of natural food and  
294 food products can induce apoptosis in various tumor  
295 cells. There is strong evidence supporting the positive  
296 role of some natural materials and medicinal plants in  
297 oncology and their ability affect all phases of  
298 tumorigenic process. Therefore, it is important to screen  
299 the natural products either as crude extracts or as  
300 isolated components for apoptotic properties to identify  
301 potential anti-cancer compounds. Over 60% anti-cancer  
302 agents currently used are derived from natural sources,  
303 including plants, marine organisms and micro-



**Fig 1.** Cytotoxicity studies aqueous extract of *C.melo* using MTT assay



**Fig 2.** Cytotoxicity studies aqueous extract of *C.melo* using Neutral red assay

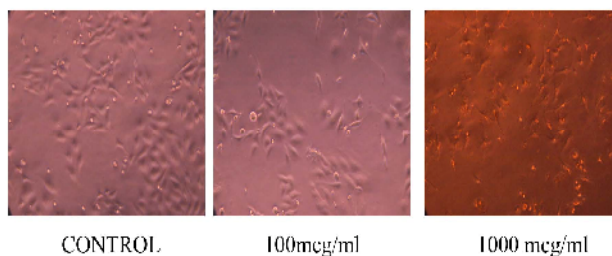


Fig 3. Photograph of PC-3 cell line: i) control received vehicle, ii) *C.melo* at a conc.100 $\mu$ g/ml and iii) *C.melo* at a conc.1000  $\mu$ g/ml

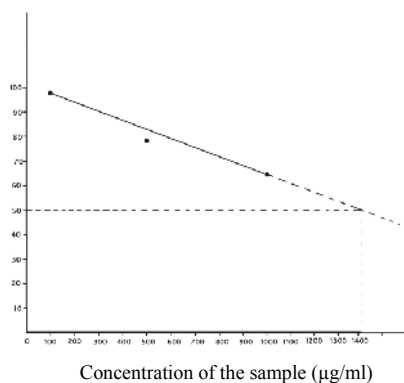


Fig 4. IC<sub>50</sub> value of aqueous extract of *C.melo* using MTT assay

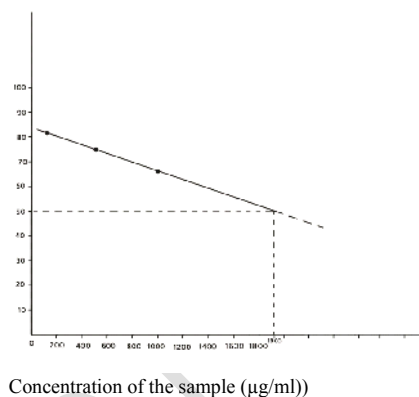


Fig 5. IC<sub>50</sub> value of aqu. extract of *C.melo* using Neutral red assay

304 organisms and they offer an opportunity to study the 333 2.  
 305 molecular mechanisms of tumorigenesis. 334  
 306 Cucurbitaceae plants are highly useful as they have 335  
 307 good potential against many health ailments. In the 336  
 308 present study, the phytochemical screening of the 337 3.  
 309 aqueous extracts of plant sample revealed the presence 338  
 310 of alkaloids and flavonoids and phytosterols [9]. These 339  
 311 phytoconstituents may be responsible for various 340 4.  
 312 activities. Flavonoids are diverse family of compounds 341  
 313 commonly found in fruits, vegetables and honey. 342  
 314 Flavonoids are generally safe and associated with low 343 5.  
 315 toxicity, making them ideal candidates for cancer 344  
 316 chemopreventive agents. MTT results and neutral red 345  
 317 uptake assay confirms dose-dependent anti-proliferative 346 6.  
 318 effect of crude aqueous extract of *Cucumis melo* on 347  
 319 prostate cancer cell lines. As the dose of the extract 348 7.  
 320 increases, number of viable cells decreases and 349  
 321 confirms the cytotoxic activity. 350  
 322 It is concluded that the aqueous extract of *C. melo* 351  
 323 was found to possess dose-dependent cytotoxic activity 352 8.  
 324 on metastatic human prostate cancer cell lines PC-3 353  
 325 Further studies are warranted to explore the anticancer 354  
 326 effect of *C. melo* and also the active principles could be 355 9.  
 327 isolated and investigated. 356  
 357  
 358

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**Effect of *cucumis* on prostate cancer**

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