ISSN: 2320-1983 Print ISSN: 2320-1991 Online Volume 2 Issue 2

Applied Cell Biology

Regular Paper

ACB, 2(2), 2013 [52-57]

In vitro antimitotic, antiproliferative and DNA fragmentation assay of ethanol extract of *Carmona retusa* (Vahl.) Masam

C.P.Chandrappa[1], M.Govindappa[1]*, N.V.Anil Kumar[2]

[1]Department of Biotechnology, Shridevi Institute of Engineering and Technology, Tumkur 572106, Karnataka, (INDIA)

[2]Department of Chemistry, Manipal Institute of Technology, Manipal University, Karnataka, (INDIA)

E-mail : dravidateja07@gmail.com

Received: 2nd March, 2013 ; Accepted: 13th May, 2013

ABSTRACT

Carmona retusa leaf decoction is being used to treat cough and stomach ache root as antidote. This plant is also reported antibacterial, anti-inflammatory, anti-analgesic and antidiabetic activity. Hence, our present study was performed to evaluate *in vitro* cytotoxicity, antiproliferative, antimitotic and DNA fragmentation assays. Fresh stem of *Carmona retusa* was collected from Hemagangothri Campus, Hassan district of Karnataka, India. Flask extraction procedure was adapted for extraction with alcohol. Alcohol extract was subjected to *in vitro* anti-mitotic, anti-proliferative activity and DNA fragmentation assay. The alcoholic extract of *C. retusa* has shown significant antimitotic and antiproliferative activity. The mitotic index was found to be 12.5 and 12.7 mg/mL respectively, which was near to standard, lapachol 12.2. Based on the results obtained, it is concluded that *Carmona retusa* may be the good candidate for the treatment of cancer as plant extracts have shown anti-mitotic and anti-proliferative activity *A. cepa* and yeast respectively.

KEYWORDS

Carmona retusa;
Antimitotic;
Allium cepa root inhibition;
Antiproliferative;
DNA fragmentation.

INTRODUCTION

It is well known that the use of plants as a therapeutic material due to their chemical substances of medicinal value is common all over the world[1]. A wide variety of anti-cancer drugs exhibit cytotoxic effect by interfering with cell-cycle kinetics. These drugs are effective against cells that are proliferating and produce cytotoxic effect either by damaging the DNA during the S-phase of the cell cycle or by blocking the formation of the mitotic spindle in M-phase[2]. The general principles of the mechanisms of mitosis are best and most easily studied in the actively growing regions of plants such as a shoot or root apex[3]. *Allium cepa* L root tip meristem have been widely used for the evaluation of cytotoxicity, anti-mitotic, genotoxicity, antimutagenic and antioxidant activity by employing the growing roots of *Allium cepa*[4]. *Carmona retusa* leaf decoction is being used to treat cough and stomach ache root as antidote[5]. This plant is also reported antibacterial, anti-inflammatory, anti-analgesic and antidiabetic activity[5,6]. Hence, our present study was performed to evaluate *in vitro* cytotoxicity, antiproliferative, antimitotic and DNA fragmentation assay of alcoholic extract of stem of *Carmona retusa* (Vahl.) Masam using *Allium cepa* L root tip cells and yeast (*Saccharomyces cerevisia*).

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MATERIALS AND METHODS

Collection of plant materials

Fresh stem of *Carmona retusa* was collected from Hemagangothri Campus, Hassan district of Karnataka, India. The collected plant was authenticated with herbarium, Government Ayurvedic College, Mysore, India and Department of Studies Botany, Mysore University, Mysore. The collected stem was cleaned with deionized water and dried under shade for two weeks at room temperature. Dried stem was grounded and filtered using 0.3mm mesh. The stem powder was stored in air tight container and maintained at 4°C until use.

Preparation of sample

Solvent system used for the extraction was alcohol. Flask extraction procedure was adapted for extraction. 25 grams of the powdered stem sample was soaked in the conical flask containing solvent, wrapped with aluminum foil and placed in shaker for 48 hours at 120-130 rpm[7].

Antimitotic activity

Method adopted by Shweta *et al*[8] was used for determination of antimitotic activity using *Allium cepa* root with slight modification. *Allium cepa* were collected from Tumkur vegetable market. *Allium cepa* bulbs were sprouted in water for 24 h at room temperature. The uniform root tips of *Allium cepa* were selected for the study. These roots were dipped in the extract (10 mg/mL and 5 mg/mL) for 48h. Water was used for dilution and lapachol was used as a standard for study. After 48h, the root tips were fixed in the fixing solution of acetic acid and alcohol (1:3). Squash preparation was made by staining with acetocarmine stain. Morphology and the number of the cells were observed under microscope (40X). In all 350-400 cells were counted and cells manifesting different stages of mitosis i.e., interphase and prophase (P), metaphase (M), anaphase (A) and telophase (T) were recorded. The mitotic index was calculated using the following formula[8,10].

$$\text{Mitotic index} = \frac{P + M + A + T}{\text{Total cells}} \times 100$$

Antiproliferative activity

Evaluation of Antiproliferative activity of plant extract was done by yeast *Saccharomyces cerevisiae* model according to Shwetha *et al* [8].

Yeast inoculum preparation

The yeast was inoculated with sterilized potato dextrose broth and incubated at 37° C for 24hr and it was referred as seeded broth.

Determination of cell viability

Cell viability assay was performed with 2.5 mL of potato dextrose broth and 0.5 mL of yeast inoculums in four separate test tubes. In the first test tube distilled water, in second test tube quercetin (Sigma Aldrich) as standard (1mg/ml), in third and fourth test tubes plant extract (10mg/ml and 5mg/ml respectively) were added. All tubes were incubated at 37° C for 24hr. In the above cell suspension, 0.1% methylene blue dye was added in all tubes and they were observed under low power microscope. The no. of viable cells, those does not stain and look transparent with oval shape while dead cells get stained and appeared blue in color were counted in 16 chambers of hemocytometer and the average no. of cell was calculated. The percentage of cell viability was calculated using the formula[9].

$$\% \text{ cytotoxicity} = \frac{\text{No of dead cells}}{\text{No. of viable cell + No. of dead cells}} \times 100$$

DNA fragmentation assay

DNA fragmentation assay performed by the method of Bicas *et al*. Briefly, 0.1 mL of extract mixed with 2.5 mL potato dextrose broth and 0.5 mL of yeast inoculums. Cell suspension was incubated for 24 hours at 37° C. DNA was isolated from the treated cell suspension with Tris EDTA buffer and DNA was electrophoresed[8].

RESULTS

Antimitotic activity

Results of antimitotic activity obtained that for plant extracts (10mg/mL and 5mg/mL) and standard (lapachol – Sigma Aldrich) in different stages of cell cycle are presented in the figure 1. Results of mitotic index are presented in TABLE 1. Mitotic index were found to be 12.5 and 12.7 respectively which was close to standard, Lapachol.

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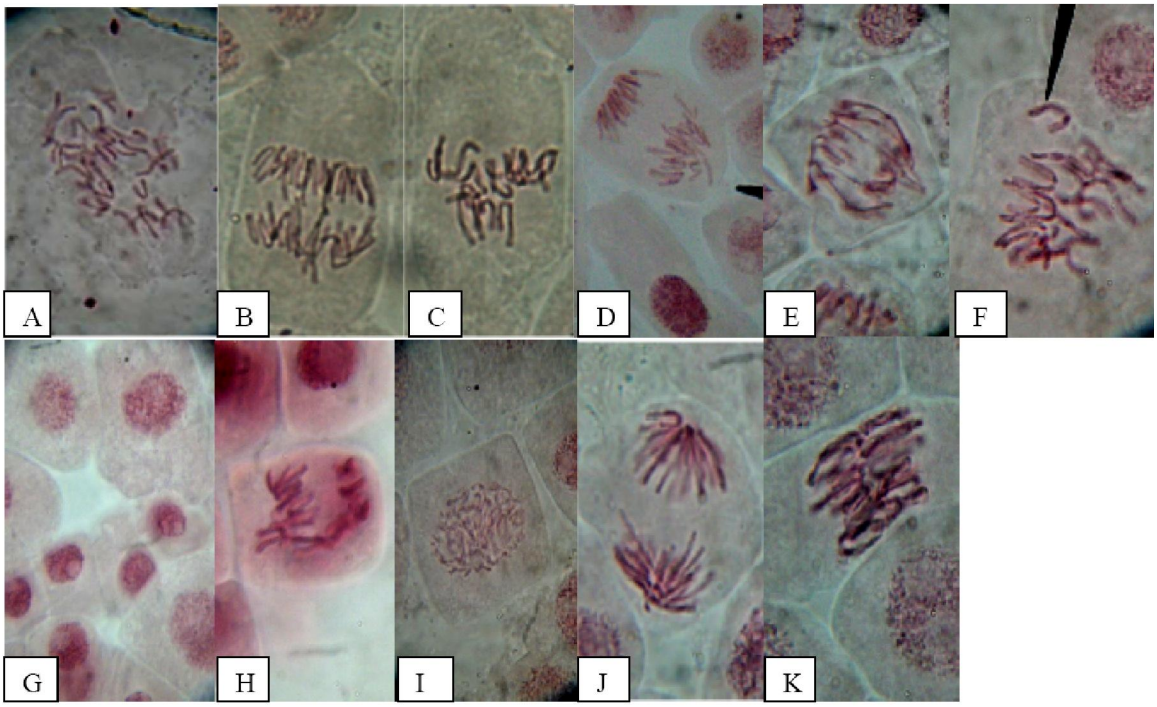

A-Chromosome Dispersion; B-Chromosomal Bridges; C-Abnormal Metaphase; D-Vigrant chromosome; E-Chromosomal Bridges; F-Deviation of chromosome; G-Abnormal Prophase surrounded with Normal Prophase (Cell Shrinkage); H- Chromosome bridge with abnormal pairs; I-Chromosome Fragmentation; J -Normal Anaphase; K-Normal Metaphase.

Figure 1 : Stages of cell division in *Allium cepa* root tip

TABLE 1 : Mitotic index in *Allium cepa* meristems with various concentrations of samples.

Extract/standard	Concentration	Mitotic index
C1	10mg/mL	12.5
C2	5mg/mL	12.7
Lapachol	1mg/mL	12.2

Antiproliferative activity

Antiproliferative activity of plant extracts have been observed in *Saccharomyces cerevisiae*. The effect of plant extracts and standards have shown in figure 2. The results of antiproliferative activity are presented in TABLE 2. During observation of treated yeast cells under microscope, we found that most of the cells were severely affected and cell debris was present on the slide and it may be due to cell necrosis. The sequence of the effect of plant extract on yeast cells have shown in figure 2.

TABLE 2 : Antiproliferative activity of plant extracts and standards.

Extract/standard	Concentration	% of cytotoxicity
C1	10mg/mL	58.3
C2	5mg/mL	54.1
Quercetin	1mg/mL	66.6

DNA fragmentation assay

A characteristic event of apoptosis is DNA fragmentation and release of nucleosome into the cytoplasm[11]. Generally cancerous cell is a mutant of normal human cell and it shows rapid growth than normal cell. DNA of the rapidly grown cancerous cell is most targets for the anticancer agents and hence the breakdown of DNA is one of the events of DNA replication which is due to inactivation of topoisomerase, a key enzyme for DNA replication[8]. The result of DNA fragmentation assay is presented in figure 3.

DISCUSSION

Root tip meristems of *A. cepa* have been utilized to evaluate the anti-mitotic and cytotoxic activity of different compounds[2]. Based on the type of organism, the drugs will effects variously. Cell treated in early interphase (G1), affects synthetic process, while later anaphase causes unequal distribution of the chromatids and chromosomal aberration causes in the gene combinations[12]. Yeast is one of most preferable model system for the evaluation of cell viability and for the conduction of DNA fragmentation assay[13].

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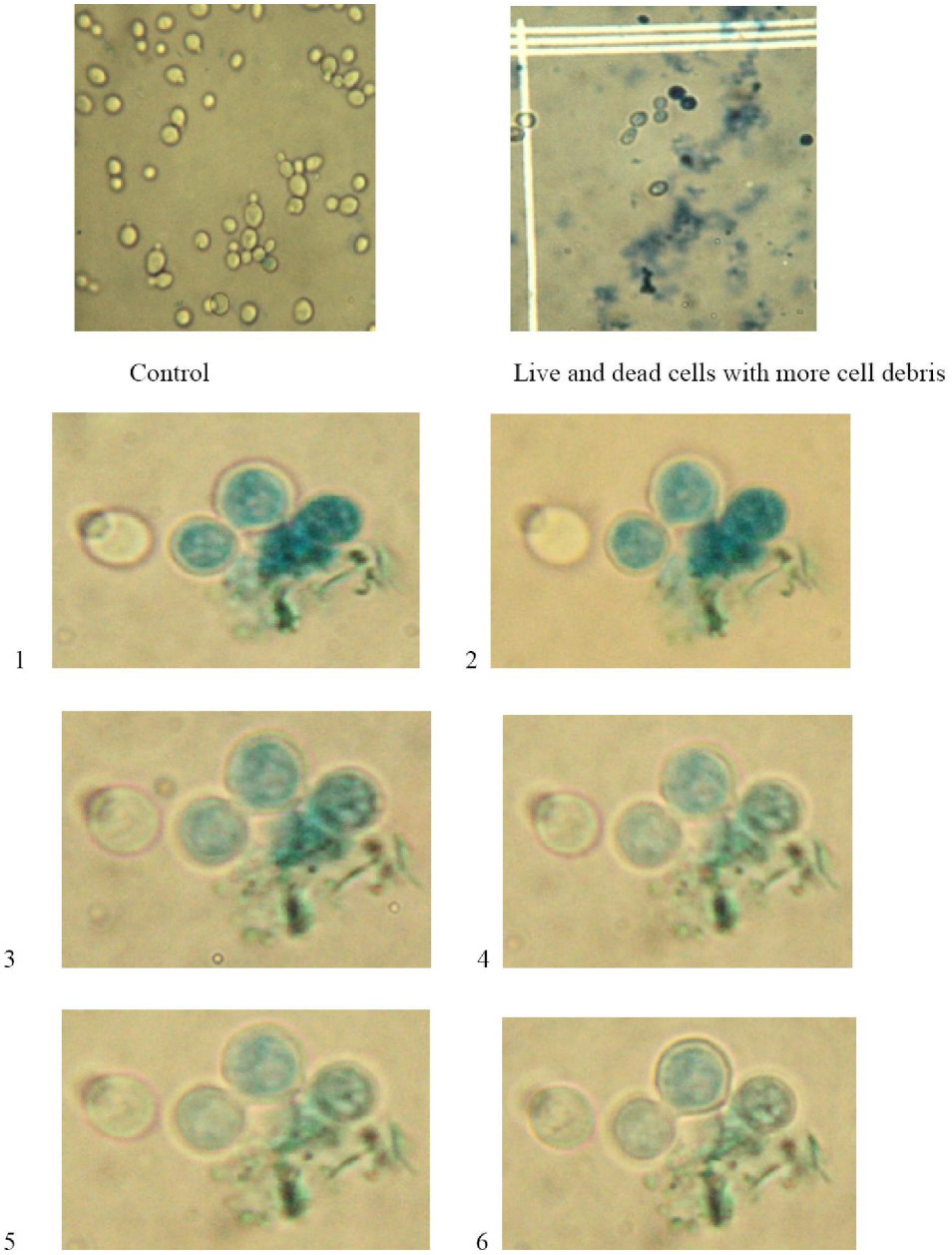

Figure 2 : The sequence of the effect of plant extract on yeast cells

In our present study, we have studied the cytotoxic, anti-mitotic, anti-proliferative and DNA fragmentation assay of alcoholic extract of *Carmona retusa*, lapachol and quercetin as standards. Anti-tumour drugs which interact with microtubules and tubulin are known to block mitosis and induce cell death by apoptosis[14]. Both lapachol and plant extract have comparably inhibited the growth of root and mitosis to a significant extent. Furthermore, anti-proliferative study and DNA fragmentation assay clearly indicates the effect of *C.retusa* in the management of cancer. Both standard and plant extracts have inhibit the DNA replication may be due to the non-function of topoisomerase-II which is one of the key enzymes in DNA replication.

Finally, it is concluded that the results of anti-mototic, anti-proliferative and DNA fragmentation assay of may be considered as an alternative mechanism of action of *C.retusa* in the inhibition of cell growth. Thus, it can assume the probable mechanism of anticancer activity of *C.retusa* due to the presence of triterpenoids[6]. It may be further concluded that the presence of flavonoids (quercetin), saponins, phenols, tannins and cardiac glycosides in the extract may contribute in cytotoxicity and inhibition of the cancerous cell growth. To evaluate the mechanism of action of plant extracts in both *in vitro* and *in vivo*,

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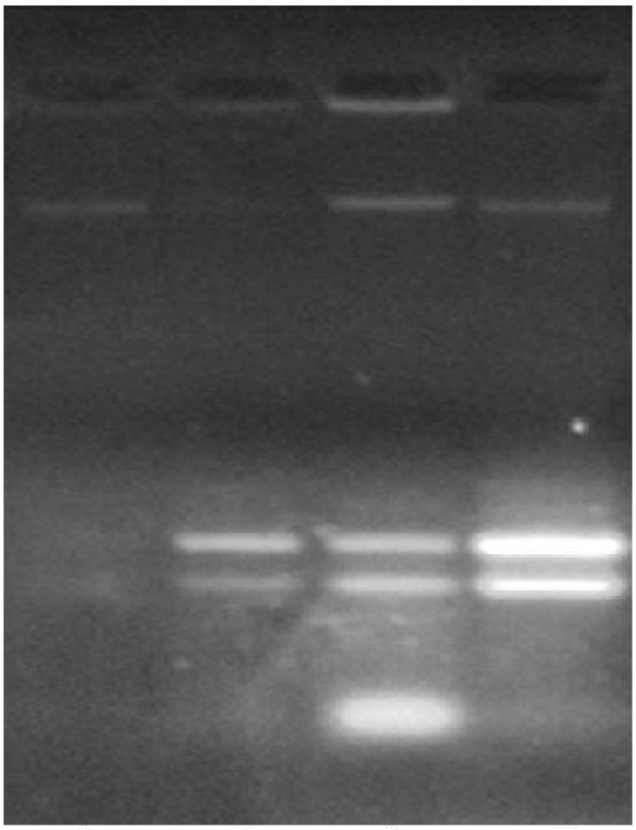

1. Distilled water 2. Quercetin 4. C1 (10mg/ml) 5. C 2 (5mg/ml)

Figure 3 : DNA fragmentation assay

further experiments need to be required.

CONCLUSION

Based on the results obtained, it is concluded that *Carmona retusa* may be the good candidate for the treatment of cancer as plant extracts have shown anti-mitotic and anti-proliferative activity *A. cepa* and yeast respectively.

ACKNOWLEDGEMENT

Authors are thankful to Dr. M.R.Hulinaykar, Managing Trustee, Sri Shridevi Charitable Trust (R.) and Dr. K.Sukumaran, Principal, Shridevi Institute of Engineering and Technology, Tumkur for providing Laboratory facilities.

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