EVALUATION OF ANTICANCER ACTIVITY OF CUCUMIS CALLOSUS AGAINST EHRlich’S ASCITES CARCINOMA BEARING MICE

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ABSTRACT

Objective: Our previous research isolated Cucurbitacin B (CuB) and ebenone leucopentaacetate (ELP) from methanolic fruit extract of Cucumis callosus (MFCC). The fruits of C. callosus (Rottl.) Cogn. (Family: Cucurbitaceae) plant have been traditionally used for antioxidant, anti-inflammatory, and antidiabetic actions. The objective of this research was to evaluate in vitro and in vivo anticancer effect of MFCC on Ehrlich Ascites Carcinoma (EAC) cell lines.

Methods: In vitro anticancer assay of MFCC and standard drug, 5-fluouracil (5-FU) was evaluated using Trypan blue and 3-(4, 5-dimethylthiazol-yl)-2, 5-diphenyl tetrazolium bromide methods. In vivo anticancer activity of MFCC and 5-FU was also performed after 24h of EAC cells (2×10^6cells/mouse) inoculation based on toxicity study for 9 consecutive days. The activity of the extract was assessed by the study of tumor volume, tumor weight, viable and non-viable cell count, hematological parameters, and biochemical estimations.

Results: The MFCC showed the direct antitumor effect on EAC cells in a dose-dependent manner with an IC_{50} value of 0.61 mg/ml. Furthermore, MFCC (350 mg/kg) exhibited significant (p<0.01) increase in tumor volume, tumor weight, and viable cell count of EAC-treated mice. Hematological profile, biochemical estimation assay significantly (p<0.01) reverted to normal level in MFCC, and 5-FU treated mice.

Conclusion: The anticancer activity of fruits of C. callosus is may be either due to the presence of CuB or/and ELP as phytoconstituent and the activity is comparable to standard drug 5-FU.

Keywords: Cucumis Callosus, Anticancer, Ehrlich Ascites Carcinoma cell lines.

INTRODUCTION

Cancer is one of the largest causes of mortality in the world in the 20th century and Hussain et al. 2012 explored the pattern and trends of cancer in Odisha. A total of 74,861 cancer inpatients were registered at Acharya Haribhushan Regional Cancer Center, Cuttack, Odisha, for the years 2001–2011 [1,2].

Nature is always the great contributor toward this goal. Plants, vegetables, and herbs have been accepted as a source of cancer-preventing agent. Most of the pharmaceutical sectors throughout the globe carried out research to find a lead compound from the traditional systems of medicine which can block the development of cancer in a human [3,4].

Most of the cancer chemotherapeutic agents are associated with toxicity toward normal cells and tissues. Optimal dosing of cancer chemotherapeutic agents is often limited because of severe bone marrow depression as toxicity. It is a continuing challenge to design a therapy that is safer, effective, and selective [5,6]. Modern cancer biology focused on new anticancer drug development that may act in different mechanisms. In fact, those compounds having cytotoxic or cytostatic ability against cancer cells show a potential anticancer activity [7].

Evidence suggests that phytochemicals from fruits and vegetables may play an important role in reducing diabetes and cancer [8,9]. The proposed plant part in this study is fruits of Cucumis callosus (Rottl.) Cogn. (Cucurbitaceae). Fruits are oval or elliptical in shape and having bitter pericarp with numerous small seeds (Fig. 1) [10,11]. Tribal peoples of Balasore and Baripada district, Odisha, and East Midnapore district of West Bengal use fruits of C. callosus as a vegetable, during worship and for curing diabetes, epilepsy, inflammatory disorders, and diarrhea [12]. Ehrlich Ascites Carcinoma (EAC) cells are experimental tumor models used worldwide in cancer research. In 1907, Paul Ehrlich discovered this tumor in the mammary gland of a white mouse, and the tumor was named after him. It is a rapidly growing carcinoma with very aggressive behavior and is able to grow in almost all strains of mice. In ascites form, it has been used as a transplantable tumor model to investigate the antitumor effects of several substances [13,14].

This research evaluated the anticancer activity of C. callosus against Ehrlich’s ascites carcinoma bearing mice. Our previous research isolated Cucurbitacin B (CuB) and ebenone leucopentaacetate (ELP) from methanolic pericarp extract of C. callosus [15]. CuB inhibits the growth of human malignant cells, both in vitro and in vivo, and has been shown to be effective against breast cancer, head, and neck squamous cell carcinoma, pancreatic cancer, hepatocellular carcinoma, osteosarcoma, and myeloid leukemia [16,17]. Consequently, natural and semi-synthetic CuBs are proposed as a promising source for the development of new drugs for the prevention and treatment of various cancers. The objective of the research was to evaluate in vitro and in vivo anticancer effect of fruits of C. callosus on EAC cell lines.

METHODS

Drugs and chemicals

5-fluouracil (5-FU), 3-(4, 5-dimethylthiazol-yl)-2, 5-diphenyl tetrazolium bromide (MTT), and Annexin V-FITC were purchased from Himedia, Mumbai, India. All other reagents used were of analytical grade obtained from Merck Specialties Private Limited, Mumbai, India.

Ethics statement

All the experiments were conducted according to norms and guidelines of CPCSEA and Institutional Animal Ethical Committee (367001/C/ CPCSEA) of University.

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Plant material and extract preparation

The C. callosus fruits (15 kg) were collected and authenticated by M. S. Mondal, Botanical Survey of India, Kolkata, India, and the voucher specimen number was CNH/1-1(196)/2007/Rich-II/1160. The methanol extract of the pericarp powder of dried fruits was prepared, and its yield was found to be 17.6% w/w.

Acute toxicity study

The LD<sub>50</sub> dose of the extract was determined by administering the extract orally to male Swiss albino mice [18].

Assay for in vitro anticancer assay

Cell culture

EAC cells were obtained from Chittaranjan National Cancer Institute (CNCI, Kolkata, India) for in vitro study. The EAC cells were maintained in vivo in Swiss albino mice by intraperitoneal transplantation of 2×10<sup>6</sup> cells per mouse after every 10 days at the Pharmacology Research Laboratory of KL Deemed to be University.

Trypan blue exclusion assay

At first, different concentrations of MFCC (0.35, 0.45, 0.55, 0.65, and 0.75) mg/mL and 5-FU (0.02, 0.12, 0.22, 0.33, and 0.44) mg/mL were prepared. 1×10<sup>5</sup> EAC cells were suspended in 0.1 mL of phosphate buffered saline (PBS, 0.2 M, and pH 7.4) and mixed with 100 mL of various aforementioned concentrations of the drug. The final concentration of drug solution was adjusted by PBS and incubated at 37°C for 3 h. After 3 h, equal quality of the drug-treated cells is mixed with Trypan blue (0.4%) and left for 1 min. It is then loaded in a hemocytometer, and the viable and non-viable count is recorded within 2 min. Viable cells do not take up color, whereas dead cells take up the color. However, if kept longer, live cells also generate and take up the color [19].

The percentage of growth inhibition is calculated using the following formula:

\[
\text{Growth inhibition(%) = } \frac{\text{Total cells} - \text{Dead cells}}{\text{Total cells}} \times 100
\]

MTT assay

The MTT assay was used for quantitative determination of viable cells. The assay was based on the conversion of the yellow tetrazolium salt-MTT to purple-formazan crystals by metabolically active cells. 96 well plates at a cell density of 2×10<sup>4</sup>/ml per well in 100 μl of RPMI 1640 were allowed to grow in a CO<sub>2</sub> incubator for 24 h (37°C, 5% CO<sub>2</sub>). The medium is then replaced and replaced by fresh medium containing different concentrations of MFCC and 5-FU for 48 h. The cells are incubated for 24–48 h (37°C, 5% CO<sub>2</sub>). Then, 20 μl MTT stock solutions (5 mg/ml in PBS) are added to each well and incubated for 5 h. The medium is removed and 200 μl dimethyl sulfoxide is added to each well to dissolve the MTT metabolic product. Then, the plate was shaken at 150 rpm for 5 min, and the optical density was measured at 560 nm.

Untreated cells (basal) are used as a control of viability (100%), and the results are expressed as percentage viability (log) relative to the control [20].

\[
\% \text{ viability} = \frac{\text{OD of test material}}{\text{OD of control}} \times 100
\]

% Inhibition= 100–(% viability)

Assay for in vivo anticancer assay

Transplantation of tumor

Ascitic fluid was drawn out from EAC tumor-bearing mouse. The viable EAC cells were counted (Trypan blue indicator) under the microscope and were adjusted at 2×10<sup>6</sup> cells/ml. All mice were injected with EAC cells suspension (0.1 ml) (ip).

Treatment schedule

30 numbers of Swiss albino mice (20–25 g) were divided into five groups. Group I kept as normal saline control (5 ml/kg bw, oral) and Group II kept as EAC control (2×10<sup>6</sup> cells/mouse, ip). After 24 h of EAC transplantation animals in Groups III and IV administered MFCC (350) and (450) mg/kg b.w; oral respectively; Group V administered 5-FU (30 mg/kg bw, oral) once daily for 9 consecutive days. After administration of the last dose, 6 mice from each group were kept fasting for 18 h and blood was collected by cardiac puncture for the estimation of hematological and biochemical parameters. Antitumor activity of MFCC was assessed by observation of changes with respect to the parameters as per Haldar et al. 2010 [13].

Evaluation of apoptosis using fluorescence-activated cell sorting (FACS)

To understand the nature of cell death, we utilized double labeling techniques using annexin-V-FITC/PI to distinguish between apoptotic and necrotic cells (Fig. 2).

RESULTS

Acute toxicity study

The LD<sub>50</sub> dose of MFCC was found to be 3500 mg/kg, b.w, p.o. in mice.

In vitro anticancer assay

The in vitro anticancer assay of the MFCC showed a direct cytotoxic effect in a dose-dependent manner. As the concentration of the drug increased, the cytotoxicity also increased. Average 50% of the cytotoxicity (IC<sub>50</sub>) was observed at the concentration of 0.61 mg/mL for MFCC and 0.346 mg/mL for 5-FU (Fig. 3).

In vivo anticancer assay

Antitumor activity of MFCC and 5-FU against EAC tumor-bearing mice was assessed by tumor volume, tumor weight, cell count (viable and non-viable), mean survival time, and percentage increase in lifespan. The tumor volume, tumor weight, and viable cell count were found to be significantly increased, and non-viable cell count was significantly decreased in EAC control animals when compared with normal control animals (Table 1). Administration of MFCC at the doses of 350 and 450 mg/kg significantly decreased the tumor volume and viable cell count. Non-viable cell count was significantly higher in MFCC treated animals when compared with EAC control animals.

There was an increased level of white blood cell (WBC) and decreased the level of hemoglobin (Hb) and red blood cell (RBC) in EAC control animals when compared with normal control (Table 2). There was a reduction in mean survival time, and percentage increase in lifespan.

Evaluation of apoptosis using fluorescence-activated cell sorting (FACS)

To understand the nature of cell death, we utilized double labeling techniques using annexin-V-FITC/PI to distinguish between apoptotic and necrotic cells (Fig. 2).

% viability = \frac{OD of test material}{OD of control} \times 100

% Inhibition= 100–(% viability)

Assay for in vivo anticancer assay

Transplantation of tumor

Ascitic fluid was drawn out from EAC tumor-bearing mouse. The viable EAC cells were counted (Trypan blue indicator) under the microscope and were adjusted at 2×10<sup>6</sup> cells/ml. All mice were injected with EAC cells suspension (0.1 ml) (ip).
Table 1: Effect of MFCC on tumor volume, tumor weight, total cell count, viable and non-viable cell count, MST and %ILS in EAC bearing mice

| Parameters                  | EAC control | EAC+MFCC (350 mg/kg) | EAC+MFCC (450 mg/kg) | EAC+5-FU (30 mg/kg) |
|-----------------------------|-------------|----------------------|----------------------|--------------------|
| Tumor volume (ml)           | 2.75±0.23   | 1.72±0.16*           | 0.88±0.14*           | 0.65±0.15*         |
| Tumor weight (g)            | 3.16±0.14   | 1.8±0.06*            | 0.92±0.07*           | 0.61±0.03*         |
| Total cell (*10^6 cell/ml)  | 9.2±0.43    | 3.7±0.31*            | 1.04±0.08*           | 0.6±0.08*          |
| Viable cell (*10^6 cell/ml) | 8.22±0.42   | 3.7±0.31*            | 1.04±0.08*           | 0.6±0.08*          |
| Non-viable cell (*10^6 cell/ml) | 0.27±0.03 | 1.3±0.08*            | 2.65±0.07*           | 3.18±0.06*         |
| Viable cell (%)             | 94.92       | 75.00                | 28.68                | 14.65              |
| Nonviable cell (%)          | 4.07        | 26.00                | 74.43                | 85.25              |
| MST (days)                  | 18.00       | 27.50                | 33.00                | 38.00              |

Values are mean±SE from 6 observations in each group. One-way ANOVA between EAC control group and treated groups followed by Dunnett’s test. *p<0.05; **p<0.01. MST: Median survival time; %ILS: Percentage increase life-span; MFCC: Methanolic fruit extract of Cucumis callosus; EAC: Ehrlich Ascites Carcinoma; SE: Standard error; ANOVA: Analysis of variance

FACS

Our flow cytometry data revealed that, in comparison with control untreated EAC cells (Fig. 4), MFCC at the doses of 350 and 450 mg/kg increases the apoptosis level by 12.7 and 14.8%, respectively.

Statistical analysis

The data expressed as the mean±standard error were statistically analyzed using one-way ANOVA followed by Dunnett's post hoc test by GraphPad Prism software, version 5. p<0.05 was considered significant and p<0.01 as highly significant.

DISCUSSION

Our previous research evaluated hypoglycemic, antioxidant, and antihyperlipidemic potential of C. callosus fruit and also, proved that many plants belonging to Cucurbitaceae family. CuB, D, E, I, and their derivatives have been studied for their anticancer activities [26]. Miliato et al. 2012 demonstrated in vitro and in vivo anticancer properties of Cucurbitacin isolated from Cayaponia racemosa [27]. The CuB exhibited strong cytotoxic effects against breast cancer cells in a dose-dependent manner and was shown to prominently alter the cytokine/leukocyte network of breast cancer cells, inducing rapid morphological changes and improper polymerization of the microtubule network [28]. Furthermore, scientists discovered that CuB inhibited the tyrosine phosphorylation of STAT3, STAT5, and JAK2 in pancreatic cancer cell lines (Panc-1 and MiaPaCa-2) in vitro and in Panc-1 xenografts in vivo. Inhibition of the JAK-STAT pathway affected various downstream targets involved in pro-growth signaling (e.g. c-myc, cyclins, and survivin) and apoptosis (e.g. p53, Bcl-xl, and Bcl-2) [29,30]. The cytotoxic effect of MFCC against EAC cells is probably due to downregulation of JAK and STAT proteins in EAC cells. The apoptotic mechanism was observed by cell-cycle analysis using FACS.

In the early stages of apoptosis, the cell membrane is still intact and impermeable to DNA binding dye PI. However, Annexin-V binds specifically with phosphatidylserine is translocated to the extracellular leaflet of the membrane. In contrast, during necrosis, because the cell membrane is ruptured, these cells take up both the fluorochromes. The MFCC-induced cell death observed in this study can occur by two distinct modes - apoptosis and necrosis, which can be distinguished by morphological and biochemical features. Annexin-V-FITC/PI staining of MFCC-treated EAC cells resulted in an increase in Annexin-V/PI+ cells compared to the control (Annexin-V/PI+), indicating apoptosis as a possible mode of cell death [31,32].

CONCLUSION

The present study demonstrates that the methanolic pericarp extract for C. callosus fruits has remarkable antitumor activity against Ehrlich's ascites carcinoma cells treated mice.

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AUTHOR'S CONTRIBUTION

Conceptualized and designed the experiments: Siva Prasad Panda and Uttam Prasad Panigrahy. Performed the experiment: Siva Prasad Panda and A.R. Reddy. Analyzed data: A.R. Reddy and Uttam Prasad Panigrahy. All authors read and approved the final manuscript.

Table 2: Effect of MFCC on hematological parameters in EAC bearing mice

| Parameters                  | Normal control | EAC control | EAC+MFCC (350 mg/kg) | EAC+MFCC (450 mg/kg) | EAC+5-FU (30 mg/kg) |
|-----------------------------|----------------|-------------|----------------------|----------------------|--------------------|
| RBC (cells/10^6/μl)         | 5.18±0.20      | 2.83±0.13*  | 4.04±0.24*           | 5.17±10±0*           | 6.0±10±0*          |
| WBC (cells/10^6/μl)         | 4.86±0.32      | 7.87±0.65*  | 6.12±0.23*           | 5.08±0.23*           | 6.12±0.66*         |
| Hemoglobin (g/dl)           | 12.46±0.38     | 4.60±0.28*  | 8.19±0.43*           | 10.15±0.36*          | 10.64±0.39*        |

Values are mean±SE from 6 observations in each group. One-way ANOVA between EAC control group and treated groups followed by Dunnett’s test. *EAC control group versus normal group; treated groups versus EAC control group. p<0.05; *p<0.01. MFCC: Methanolic fruit extract of Cucumis callosus; EAC: Ehrlich Ascites Carcinoma; SE: Standard error; RBC: Red blood cell; WBC: White blood cell; ANOVA: Analysis of variance.
Cucurbitacin B potently suppresses non-small-cell lung cancer

**Fig. 3:** Anticancer effect of methanolic fruit extract of *Cucumis callosus* and 5-FU on Ehrlich Ascites Carcinoma cell line by Trypan blue and 3-(4, 5-dimethylthiazol-yl)-2, 5-diphenyl tetrazolium bromide assay

**Fig. 4:** Evaluation of percentage of apoptosis for methanolic fruit extract of *Cucumis callosus* and 5-FU

**CONFLICTS OF INTEREST**

The author declared that they have no conflicts of interest.

**REFERENCES**

1. Abdullaev FI, Luna RR, Roitenburd BV, Espinosa AJ. The pattern of childhood cancer mortality in Mexico. Arch Med Res 2000;31:526-31.
2. Hussain MA, Pati S, Swain S, Prusty M, Kadam S, Nayak S, et al. Pattern and trends of cancer in Odisha, India: A retrospective study. Asian Pac J Cancer Prev 2012;13:633-6.
3. Abdullaev FI, Inc. Plant-derived Agents Against Cancer, Gupta SK, editors. Pharmacology and Therapeutics in the New Millennium. New Delhi: Narosa Publishing House; 2001. p. 345-54.
4. Patwardhan B, Vaidya AD, Chorghade M. Ayurveda and natural products drug discovery. Curr Sci 2004;86:6.
5. Dwiranay S, Gautam M, Patwardhan B. Cytoprotection and immunomodulation in cancer therapy. Curr Med Chem Anticancer Agents 2004;4:479-90.
6. Tripathy KD. Anticancer drugs. Essential Medical Pharmacology. 6th ed. New Delhi: Jaypee Brother’s Medical Publishers (P) Ltd.; 2007. p. 820.
7. Ramos AA, Prata-Sena M, Castro-Carvalho B, Dethoup T, Buttachon S, Kijooa A, et al. Potential of four marine-derived fungi extracts as antiproliferative and cell death-inducing agents in seven human cancer cell lines. Asian Pac J of Trop Med 2015;8:798-806.
8. Bhattacharyya VA, Graefe U, Kohler C, Veit M, Derendorf H. Pharmacokinetic and bioavailability of herbal medicinal products. Phytomedicine 2002;9:1-33.
9. Hung HC, Joshiprua KJ, Jiang R, Hu FB, Hunter D, Smith-Warner SA, et al. Fruit and vegetable intake and risk of major chronic disease. J Natl Cancer Inst 2004;96:1577-84.
10. Rathore M. Nutrient content of important fruit trees from arid zone of Rajasthan. J Hortic For 2009;1:103-8.
11. Raut M. Glimpses of Nature Series (No. 4) our Monsoon Plants, Bombay Natural History Society. Bombay, India; 1959.
12. Trivedi PC. Medicinal plants: Traditional knowledge. In: Sharma UK, Gogoi J, editors. Ethno-Medico-Botany of some Sacred Plants of Dhemaji District of Assam. New Delhi: IK International Pvt. Ltd.; 2006. p. 102.
13. Haldar PK, Kar B, Bala A, Bhattacharya S, Mazumder UK. Antitumor activity of Сансверія рошбігана rhizome against Ehrlich ascites carcinoma in mice. Pharm Biol 2010;48:1337-43.
14. Islam K, Ali SM, Jesmin M, Khanam JA. In vivo teratogenic effect of the mixture using the Zebrafish embryo developmental assay. Cancer Biol Med 2012;9:242-7.
15. Panda SP, Sarangi AK, Panigrahy UP. Isolation of cucurbitacin-B from *Cucumis callosus* and its hypoglycemic effect in isolated rat enterocytes. Int J Pharm Pharm Sci 2018;10:123-9.
16. Kausar H, Munagala R, Bansal SS, Aqil F, Vadhanan MV, Gupta RC, et al. Cucurbitacin B potently suppresses non-small-cell lung cancer growth: Identification of intracellular thiols as critical targets. Cancer Lett 2013;332:35-45.
17. Guo J, Wu G, Bao J, Hao W, Lu J, Chen X, et al. Cucurbitacin B induced ATM-mediated DNA damage causes G2/M cell cycle arrest in a ROS-dependent manner. PLoS One 2014;9:e88140.
18. Panda SP, Haldar PK, Bera S, Adhikary S, Kandar CC. Anti-diabetic and antioxidant activity of *Swietenia mahagoni* in streptozotocin-induced diabetic rats. Pharm Biol 2010;48:974-9.
19. Bala A, Kara B, Haldar P, Mazumder U, Bera S. Evaluation of anticancer activity of *Cissus gynandra* on Ehrlich’s ascites carcinoma treated mice. J Ethnopharmacol 2010;129:131-4.
20. Mossman T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
21. Chakraborty M, Majumder S, Mazumder S, Das S, Haldar PK. The anti-diabetic, antioxidant and anti-hyperlipidemic activity of *Cucumis callosus* rhizome against streptozotocin-induced diabetic rats. Curr Pharm Sci 2016;7:1978-84.
22. Prasad SB, Giri A. Antitumor effect of cisplatin against murine ascites carcinoma in mice. Iranian J Pharmacol Ther 2008;13:2340-69.
23. Rajeshwar Y, Gupta M, Mazumder UK. Antitumor activity and antiproliferative and cell death-inducing agents in seven human cancer cell lines. Asian Pac J of Trop Med 2015;8:798-806.
24. Miura K, Kinouchi M, Fujibuchi W, Naitoh T, Ogawa H, et al. Cucurbitacin B inhibits human breast cancer cell proliferation through disruption of microtubule polymerization and nucleophosmin/B23 binding. Mol Pharm 2010;7:2122-30.
25. Militao GC, Dantas IN, Ferreira PM, Alves AP, Chaves DC, Monte FJ, et al. Antioxidant and anticancer properties of cucurbitacin isolated from *Cucumis callosus* and *Sansevieria roxburghiana* rhizome. Cancer Biol Med 2012;9:1717-30.
26. Kausar H, Munagala R, Bansal SS, Aqil F, Vadhanan MV, Gupta RC, et al. Cucurbitacin B potently suppresses non-small-cell lung cancer growth: Identification of intracellular thiols as critical targets. Cancer Lett 2013;332:35-45.
27. Thoennissen NH, Iwanski GB, Doan NB, Okamoto R, Lin P, Abbassi S, et al. Cucurbitacin B inhibits human breast cancer cell proliferation through disruption of microtubule polymerization and nucleophosmin/B23 binding. Mol Pharm 2010;7:2122-30.
28. Duangmano S, Sae-Lim P, Suksamrarn A, Domann FE, Patmasiriwat P. Cucurbitacin B inhibits human breast cancer cell proliferation through disruption of microtubule polymerization and nucleophosmin/B23 translocation. BMC Complement Altern Med 2012;12:185.
29. Tripathy KD. Anticancer drugs. Essential Medical Pharmacology. 6th ed. New Delhi: Jaypee Brother’s Medical Publishers (P) Ltd.; 2007. p. 820.
30. Brantley EC, Benveniste EN. STAT-3: A molecular hub for signaling pathways and potentiate cytotoxic effects of gemcitabine on pancreatic cancer cells. Cancer Res 2009;69:5876-84.
31. Mukherjee C, Paul S, Kundu R. Comparative evaluation of ant proliferative activity of *Solanum nigrum* methanolic and aqueous extract on HELA, SIHA and C33A cells. Int J Pharm Pharm Sci 2015;7:320-4.

32. Kumar Y, Periyasamy L. GC-MS analysis and *in-vitro* cytotoxic studies of *Bixa orellana* seed extract against cancer cell line. Int J Pharm Pharm Sci 2016;8:408-13.