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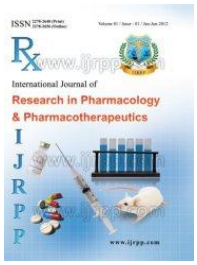

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Evaluation of *in vitro* anticancer activity of *cucumis sativus* linn leaves

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ABSTRACT

Qualitative analysis of the extract revealed that it contains Glycosides, Alkaloids, Tannins, Proteins and amino acids, Phytosterol and Steriods, Terpenoids, Saponins. The anticancer activity of ethanolic extract of *Cucumis sativus* Linn (EECS) was studied on the Various cell line HeLa, HepaG2 by using MTT Assay method. The EECS at dose 62.5 µg, 125 µg, 250 µg, 500 µg, produced a significant anticancer activity against HeLa and HepG2 cancer cell lines.When compare the % cell inhibition of HeLa and HepG2, HepG2 is giving more significant activity than HeLa. It shows that triterpenoids present in extract may be possibly responsible for the anticancer activities.

KEYWORDS: *Cucumis sativus*, Anticancer, Liver Cancer, Cervical Cancer.

INTRODUCTION

Cancer is a general term applied of series of malignant diseases that may affect different parts of body. These diseases are characterized by a rapid and uncontrolled formation of abnormal cells, which may mass together to form a growth or tumor, or proliferate throughout the body, initiating abnormal growth at other sites. If the process is not arrested, it may progress until it causes the death of the organism. The main forms of treatment for cancer in humans are surgery; radiation and cancer chemotherapeutic agents.[1] Cancers that start in the liver are called liver cancer. To understand liver cancer, it helps to know about the normal structure and function of the liver. The liver is made up mainly of cells called *hepatocytes*. It also contains other types of cells, including cells that line its blood vessels and cells that line small tubes in the liver called *bile ducts*. The bile ducts extend out of the liver and carry bile from the liver to the gallbladder or directly to the intestines. In recent years, a lot of effort has been applied to the synthesis of potential anticancer drugs. Many hundreds of chemical variants of known class of cancer chemotherapeutic agents have been synthesized but have a more side effects. A successful anticancer drug should kill or incapacitate cancer cells without causing excessive damage to normal cells. This is difficult, or perhaps impossible, to attain and is why cancer patients frequently suffer unpleasant side effects when under-going treatment. Synthesis of modifications of known drug continues as an important aspect of research. However, a waste amount of synthetic work has given relatively small improvements over the

prototype drugs. There is a continued need for new prototype-new templates to use in the design of potential chemotherapeutic agents: natural products are providing such templates. Recent studies of tumor-inhibiting compound of plant origin have yielded an impressive array of novel structures[1]. Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicine, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance[2]. Due to the prevalence, morbidity, and mortality of the malignant diseases, they represent significant medical, social and financial burden on the society. At present the pharmacological therapy of cancer is limited to symptomatic treatments that do not alter the course of the underlying disease. To date, specific pharmacologic agents that can be used in the management of cancer are lacking and yet a rather scanty number of potential therapeutic agents are under clinical trials. Several Indian medicinal plants have been extensively used in the Indian traditional system of medicine for the treatment of cancer diseases due to their Potent pharmacological activity, Low toxicity and less time, Economic viability and renewable sources, Long history of use, better patient tolerance, public acceptance, Cultivation and processing conditions environmental friendly, Avoid Environmental pollution by the chemical industry[3-11]. *Like* other herbs, its anticancer activity in various cell line HeLa, HepaG2 by using MTT Assay method is debatable & its anticancer profile has not been investigated before. To overcome the disadvantage and develop a new therapeutic agents from nature. Plant Name: *Cucumis sativus* Linn, Family: Cucurbitaceae, Kingdom: Plantae, Order: Cucurbitales, Genus: *Cucumis*, Species: *C. Sativus.* Vernacular Names are Tamil: Mullu Vellarikkai, Bengal: Khira, Hindi: Khira, English: Cucumber, Sanskrit: Sakusa, Telugu: Dozakaya, Malayalam: Mullan vellari. North India is considered to be the original home of the species, Cultivated in all parts of India, and in warm and temperature countries throughout the world. A hispidly hairy climber with membranous deeply cordate angled or shallowly 3 – 5 lobed leaves about 11.5 cm diameter. The larger up to 14 to 15cm both sides hairy with softish hairs but the upper with thickened bases and the ribs beneath scabrid, or hispid margin denticulate, terminal lobe. Some lanceolate and basal lobes sometimes subhastate. Petiole – (5 – 10) cm flowers yellow 1.8 – 2.5cm diameter.The fruit contain an enzyme, erepsin, vitamin B1 and Ascorbic acid proteolytic enzyme, rutin, oxidase, succinic acid and maleic dehydrogenases. The seeds glucosides including cucurbitasides alpha and beta amyrin, sitosterols. The leaves contain free cucurbitasides B and C, ferredoxin, alpha- spinasterol. Traditionally this plant is used for headache, the seeds are cooling and diuretic, seeds used in dysuria, irritation of the urinary tract, cystitis. Reduces specific gravity of urine and also used for tapeworms. The fruit juice of this plant is used as a nutritive and as a demulcent in anti- acne lotions. The leaves, boiled and mixed with cumin seeds, roasted and powdered, and administered in throat infections[11-33]

MATERIALS AND METHODS

COLLECTION & AUTHENTICATION OF PLANT

The plant leaves of *Cucumis sativus* Linn was collected from surrounding areas of Komarapalayam and Sankagiri, Namakkal District, Tamilnadu, India & authenticated by Mr. G.V.S. Murthy, scientist F, Botanical survey of India, Coimbatore, Tamilnadu (No.BSI/SRC/5/23/2011-12/Tech).

EXTRACTION PROCEDURE

The leaves of *Cucumis sativus* Linn were dried under shade, mixed together and then made in to a coarse powder with a mechanical grinder. The powder was passed through sieve no.40 and stored in an airtight container for further use. The dried powder material (150gm) was defatted with petroleum ether (60-80^{o}C) to remove waxy substances and chlorophyll, which usually interfere in the isolation of phytoconstituents. The marc after defatted with petroleum ether was dried and extracted with ethanol (99.9%v/v) in a Soxhlet extractor for 72 hr. The solvent was then distilled off and the resulting semisolid mass was dried in a vacuum evaporator to get a yield of 5%w/w.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

The extract of *Cucumis sativus* Linn was subjected to qualitative tests for the identification of various plant constituents like alkaloids, glycosides, tannins, saponins, proteins and amino acids, phytosterol and steroids,and terpenoids.[33-38]

PHARMACOLOGICAL STUDIES

IN VITRO ANTICANCER ACTIVITY

The human cervical cancer cell line (HeLa) and human liver cell carcinoma cell line (HepG2) were obtained from National Centre for Cell Science (NCCS), Pune, and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). All cells were maintained at 37 ºC, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

CELL TREATMENT PROCEDURE

The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a haemocytometer and diluted with medium with 5% FBS to give final density of $1x10^5$ cells/ml. one hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the extracts and fractions. They were initially dissolved in neat dimethyl sulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. One hundred microlitres per well of each concentration was added to plates to obtain final concentrations of 500, 250, 125, 62.5 and 31.25 µg/ml. The final volume in each well was 200 µl and the plates were incubated at 37^0C, 5% CO_2, 95% air and 100% relative humidity for 48h. The medium containing without samples were served as control. Triplicate was maintained for all concentrations.

MTT ASSAY

MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the amount of formazan produced is directly proportional to the number of viable cells. After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

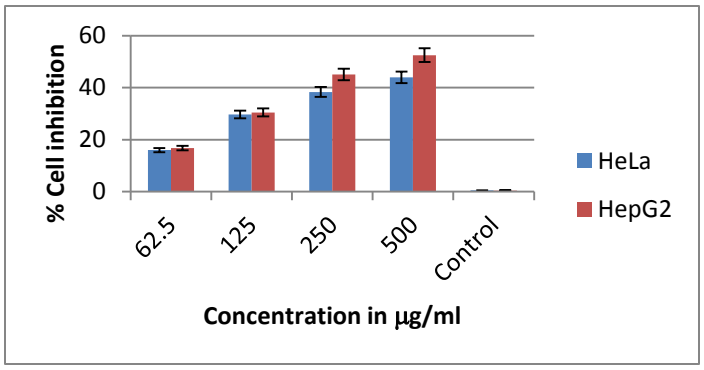
% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log_{10} concentration and IC50 was determined using Graph Pad Prism software.[39, 40]

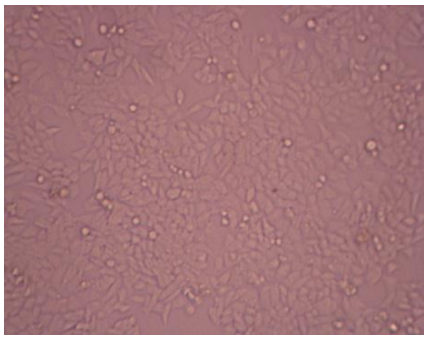
RESULTS AND DISCUSSION

The plant leaves of *Cucumis Sativus Linn* [cucurbitaceae] were powdered and extracted successfully with petroleum ether and ethanol using hot continuous Soxhlet extraction. The ethanolic extract of *Cucumis Sativus Linn* was subjected to qualitiative phytochemical screening to identify the active constituents which showed the presence of Glycosides, Alkaloids, Tannins, Proteins and amino acids, Phytosterol and Steriods, and Terpenoids, Saponins. The anticancer activity of ethanolic extract of *Cucumis Sativus Linn* was studied on the Various cell line HeLa, HepaG2 by using MTT Assay method. The EECS at does 62.5 µg, 125 µg, 250 µg, 500 µg, produced a significant anticancer activity against HeLa and HepG2 cancer cell lines. The % cell inhibition (Fig no:1&2) of HeLa was 43.93, and HepG2 was 52.46.

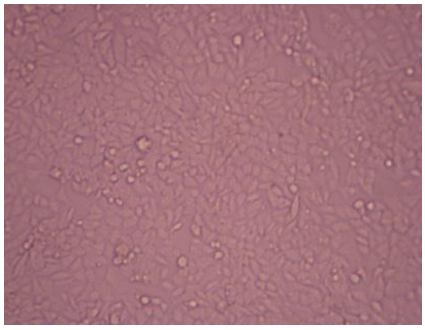
***In Vitro* Anticancer activity of EECS on Different Cell Lines: Graph-1**



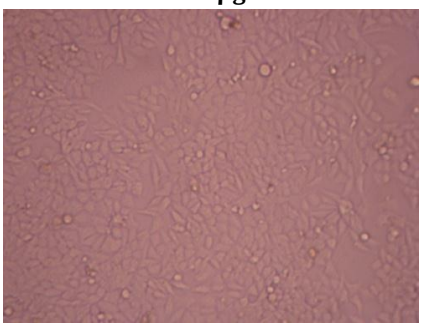
CONTROL



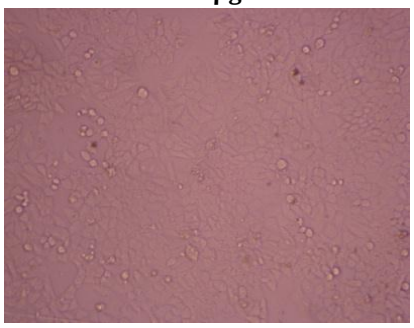
62.5µg



125µg



250µg



500µg

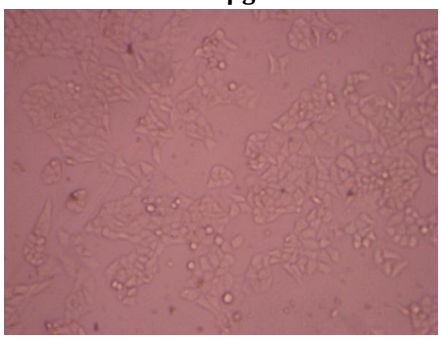


Fig. No 1. Growth inhibition diagram of HeLa cell line

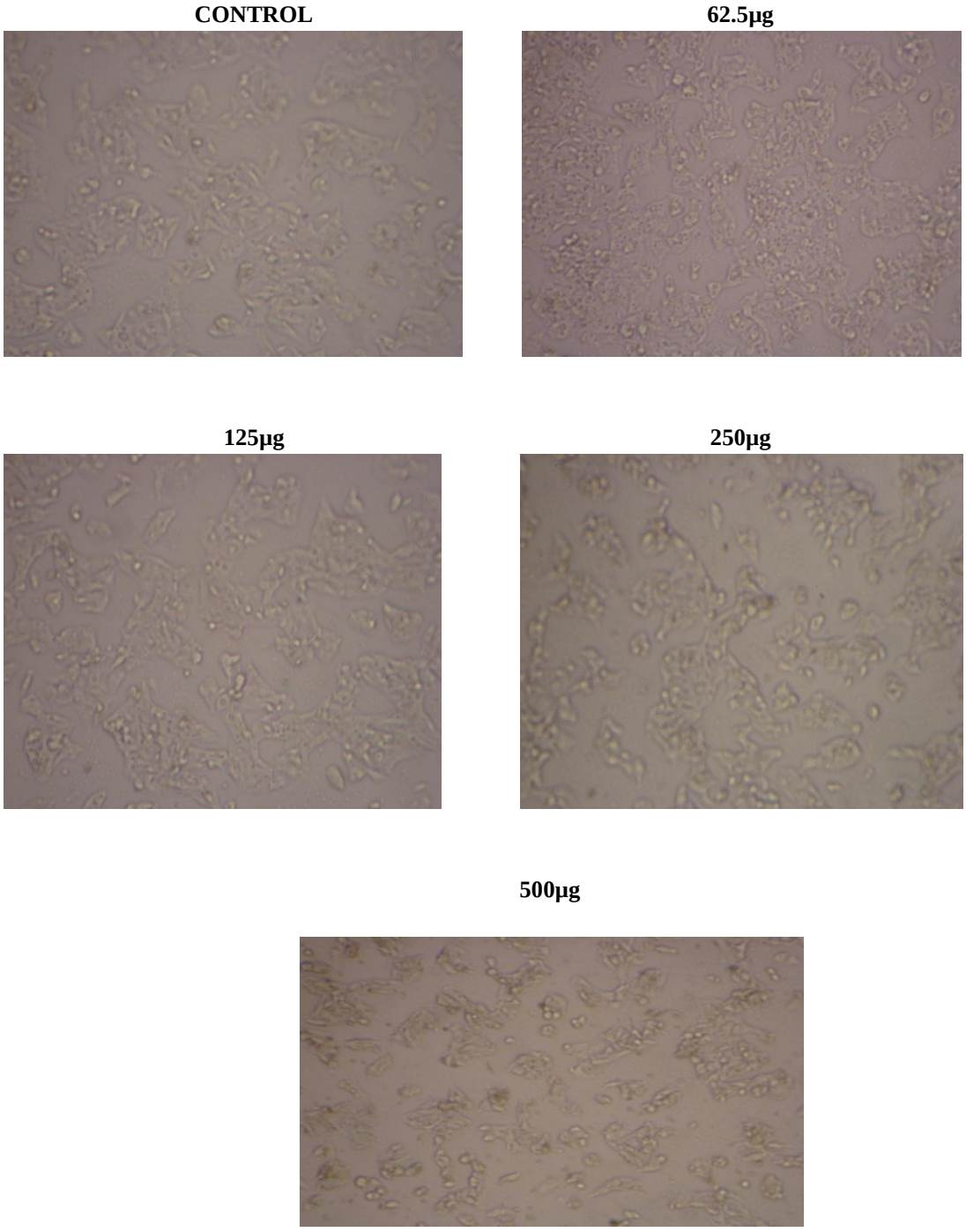


Fig. No.2. Growth inhibited diagram of HepG2 cell line

SUMMARY AND CONCLUSION

The ethanol extract of *Cucumis Sativus* Linn was evaluated for *in vitro* anticancer activity. The EECS extract at doses 62.5 µg, 125 µg, 250 µg, 500 µg produced a significant anti cancer activity against HeLa and HepG2 cancer cell lines. When compare the HeLa and HepG2, HepG2 is giving more significant activity then HeLa. It shows that triterpenoids present in extract may be possibly responsible for the anticancer activities.

REFERENCES

1. Sakarkar DM, Deshmukh VN, Ethno pharmacological Review of Traditional Medicinal Plants for Anticancer Activity, *International Journal of Pharm Tech Research*, 3:298 – 308.
2. Joy PP, Thomas J, Samuel Mathew, Baby Skaria, Medicinal plants, 1998: 3-6.
3. Kokate CK, Purhoit AP, Gokhale SB, "Pharmacognosy", Nirali Publication, Pune, 16th Edition, 2001:134, 196, 435.
4. Mohammed Ali, Text Book of Pharmacognosy, CBS Publishers and Distributors, 1st Edition, 1994:1 – 3.
5. http:/www.herpalace.com/alternative-medicine/herbal-medicine.
6. Trease and Evans, "A Textbook of Pharmacognosy", Saunder's Elsevier 15th Edition, p.3, 4, 394, 419, 470.
7. Schulz, Hansel VR & Tyler VE, Rational phytotherapy. A physicians Guide to Herbal Medicine, 4th Ed, Berlin, Springer-verlag, 2001.
8. Li L, Opportunity and challenge of traditional chinese medicine in face of the entrance to WTO (World Trade Organization). Chin. Inform. trade. *chin. Med.,* 2000: 7-8(in chinese).
9. Satio H, Regulation of herbal medicines in japan. Pharmacol. Regul. 41, 2000:.515-519.
10. Morgan K, Medicine of the Gods: Basic principles of Ayurvedic Medicine (http:// www. Compulink. Co.ut /~mandrake/ Ayurveda.htm), 2002.
11. WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems. 2004:5, 6.
12. Kirtikar & Basu, *The Indian Medicinal Plants*, vol. 2, 1993: 1144.
13. Khare CP, *Indian medicinal plants*, 2007:183.
14. Hisharo kai, Masaki baba, and Toru okuyama, Two New Megastigmanes from the leaves of *Cucumis sativus*, *Chem pharm. Bull*, 2007; 55:133-136.
15. Hideki Horie, Hidekazu ito, Katsunari ippoushi, Keiko azuma, Yoshiteru sakata and Isamu igarashi, Cucurbitacin C-Bitter Principle in Cucumber Plants, Jarq, 2007; 41: 65-68.
16. Kumar D, Kumar S, Singh J, Narener, Rashmi, Vashista BD, and Singh N, Free Radical Scavenging and Analgesic Activities of *Cucumis sativus L*. Fruit Extract, *J Young pharm*, 2010; 2(4):365-368.
17. Amand Paul C. St and Wehner Todd C, Greenhouse, Detached-leaf, and Field Testing Methods to Determine Cucumber Resistance to Gummy Stem Blight, *J Amer. Soc. Hort. Sci* 1995; 120(4):673-680.
18. Mohsen Minaiyan, Behzad Zolfaghari, Amin Kamal, Effect of Hydroalcoholic and Buthanolic Extract of *Cucumis sativus* Seeds on Blood Glucose Level of Normal and Streptozotocin-Induced Diabetic Rats, *Iranian Journal of Basic Medical Sciences* 2011; 4(5) : 436-442.
19. David M. Pharr, Steven C. Huber, and Harriet N. Sox, Leaf Carbohydrate Status and Enzymes of Translocate Synthesis in Fruting and Vegetative Plants of *Cucumis sativus L., Plant Physiol.* 1985; 77:104-108.
20. Jun Ohkawa, Naosuke Okada, Atsuhiko Shinmyo, and Mitsuo Takano, Primary structure of cucumber (*Cucumis sativus*) ascorbate oxidase deduced from Cdna sequence: Homology with blue copper proteins and tissue-specific expression, *Prac. Natl. Acad. Sci.* USA, 1989; 86:1239-1243.
21. Joysree Das, Anusha Chowdary, Subrata Kumar Biswas, Utpal Kumar Karmakar, Syeda Ridita Sharif, Sheikh Zahir Raihan and Md Abdul Muhit., Cytotoxicity and Antifungal Activities of Ethanolic and Chloroform Extracts of *Cucumis sativus* Linn (Cucurbitaceae) Leaves and Stems, *Research Journal of Phytochemistry* 2012; 1: 1819-3471.
22. Gill NS, Garg M, Bansal R, Sood S, Muthuraman A, Bali M, and Sharma PD, Evaluation of Antioxidant and Antiulcer Potential of *Cucumis sativum L*. seed Extract in Rats, *Asian Journal of Clinical Nutrition* 2009; 1:131-138.
23. Charles R. Caldwell, Estimation and Analysis of Cucumber (*Cucumis sativus L.*) Leaf Cellular Heat Sensitivity, *Plant Physiol*, 1993; 101:939-945.
24. Sudheesh S, and Vijayalakshmi NR, Role of pectin from Cucumber (*Cucumis sativus*) in modulation of protein kinase C activity and regulation of glycogen metabolism in rats, *Indian Journal of Biochemistry & Biophysics* 2007; 44:183-185.

25. Patrizia De Nisi and Graziano Zocchi, Phosphoenolpyruvate carboxylase in cucumber (*Cucumis sativus L.*) roots under iron deficiency: activity and kinetic characterization, *Journal of Experimental Botany* 1903; 55:1909.
26. Senthil V, Ramasamy P, Elaiyaraja C, and Ramola Elizabeth A, Some Phytochemical Prosperities Affected by the Infection of Leaf Spot Disease of *Cucumis sativus* (Linnaeus) Caused by Pencillium notatum, *African Journal of Basic & Applied Sciences*, 2010; 2 (3-4):64-70.
27. Jeffery F, Derr and Thomas J, Monaco, Ethalfluralin Activity in Cucumber (*Cucumis sativus*), Jstor: Weed Science 1982; 30: 498-502.
28. Anisimov MM, Logachev VV, Sil Chenko AS, and Avilov SA, Influence of Triterpene Glycosides of the Holothurian Cucumaria japonica on Root Growth in *Cucumis sativus L*. Seedlings, *Biology Bulletin* 2004; 31:505-512.
29. Serquen Felix C, Bacher J, Staub JE, Mapping and QTL analysis of horticultural traits in a narrow cross in cucumber (*Cucumis sativus L.*) using random-amplified polymorphic DNA markers, Molecular Breeding 1997;3:257-268.
30. Neelesh K, Nema, Niladri Maity, Sushil K, Chaudary, Sarkar B, Mukherjee Pulok K, Matrix metalloproteinase-1 inhibitory activity of *Cucumis sativus* lyophilized juice.
31. Ronald Pierik, Wouter Verkerke, Rens, Blom and Eric Visser, Thick Root Syndrome in Cucumber (*Cucumis sativus L.*) A Description of the Phenomenon and an Investigation of the Role of Ethylene, Annals of Botany1999; 84:755-762.
32. Solange AB, Dos Santos, Ana C, Roselino and Luci R, Bego, Pollination of cucumber, *Cucumis sativus L*. (Cucurbitales: Cucurbitaceae), by the Stingless Bess Scaptotrigona aff. Depilis Moure and Nannotrigona testaceicornis Lepeletier (Hymenoptera: Meliponini) in Greenhouses, Neotropical Entomology 2008; 37 (5): 506-512.
33. Zhujun Zhu, Guoqiang Wel, Juan Li, Qiongqiu Qian, Jingquan Yu, Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus L.*), *Plant Science*2004; 167(3);527-535.
34. Naresh Singh Gill and Manoj Rali, Evaluation of Antioxidant, Anti-ulcer Activity of 9-beta-methyl-19-norlanosta-5-ene Type Glycosides from *Cucumis sativus* seeds, *Reaserch Journal of Medicinal Plant* 2012; 6(4):309-317.
35. Harborne JB, Phytochemical Method, A Guide to modern techniques of plant Analysis, Springer (India) Pvt. Ltd, New Delhi, 3rd edition, 2005:5-16, 22.
36. Krishnaswamy NR, Chemistry of Natural Products, A laboratory hand book, Universities press India (Pvt.) Ltd, Hyderabad, 1st edition , 2003:15, 26-60, 70-73, 87-88.
37. Gurudeep R Chatwal, Sham K Anand, Instrumental methods of Chemical Analysis, Himalaya publishing house Mumbai, 5th revised edition, 2003:567.
38. Dr. Kasture AV, Dr. Wadodkar SG, Dr. Mahadik KR, Dr. More HN, Pharmaceutical Analysis, Nirali Prakashan, Pune, , 9th edition, 2003: vol II, 16.
39. Mosmann T, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *Journal of Immunological Methods*, vol. 65, 55-63.
40. Monks A, Feasibility of high flux anticancer drug screen using a diverse panel of cultured human tumour cell lines, *Journal of the National Cancer Institute*, vol. 83, 757-766