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Evaluation of bioactivity of *Cucurbita pepo* L., *Cucumis melo* L. and *Cucumis sativus* L. seed extracts

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The herbal family Cucurbitaceae are rich in nutrients as well as compounds of medicinal interest viz. taxols and microtubule inhibitors, particularly against cancer. Here, we evaluated the cytotoxic, antimitotic, and antiproliferative properties of *n*-hexane and petroleum ether seed extracts of pumpkin *Cucurbita pepo* L. (CP), Muskmelon *Cucumis melo* L. (CM) and cucumber *Cucumis sativus* L. (CS). The cytotoxic activity was determined using brine shrimp lethality bioassay, antimitotic activity was determined using *Allium cepa* root growth inhibition test and *Vigna radiata* (green gram) germination test, and antiproliferative activity was determined by cell viability assay using yeast model on the extracts. The extracts showed positive concentration dependent cytotoxicity towards brine shrimp. The LC_{50} values for *n*-hexane extracts of CP, CM and CS were 750, 250 and 500 µg/mL, respectively, and for petroleum ether extracts, the LC_{50} values were 500, 500 and 1000 µg/mL, respectively. Good root growth inhibition of *Allium cepa* was observed for both the extracts of CP, CM and CS with the efficient concentration (EC_{50}) values 500, 500, 250 ppm and 500, 250, 500 ppm, respectively. Antimitotic activity of extracts showed significant inhibition of imbibition morphologically. Percentage of inhibition for *n*-hexane extracts of CP, CM, CS were 119.9, 121.7, 108.7% and that for petroleum ether extracts of CP, CM, CS were 58, 58.41, 61.28%, respectively. Antiproliferative assay using yeast (*Saccharomyces cerevisiae*) model showed good concentration-dependent inhibition of yeast cell growth. The percentage of cell inhibition for *n*-hexane extract of CP (1, 2, 3 mg/mL) was 75, 82.7 and 95.6%, respectively. For CM, it was 75.8, 84.3 and 97.6% while that for CS, it was 72.9, 81.7 and 92.5%, respectively. The percentage of cell inhibition for petroleum ether extract of CP (1, 2, 3 mg/mL) was 70.5, 76.4 and 90.1%, respectively. For CM, it was 72.3, 78.8 and 91.9% while that for CS, it was 72.7, 82.1 and 93.1, respectively. The results have demonstrated significant cytotoxic, antimitotic and antiproliferative activity of the seed extracts of *Cucurbita pepo*, *Cucumis melo* and *Cucumis sativus*.

Keywords: Antimitotic, Antiproliferative, Brine shrimp lethality bioassay, Cucumber, Cytotoxic, Muskmelon, Pumpkin

Medicinal plants have been the most important source of life saving drugs since ancient time. Use of herbal medicine is increasing in both developing and industrialized countries[1]. Biologically active components from plants are significant source of new drugs that are likely to lead towards better treatment options for cancer[2]. Herbal medicines are mixtures of therapeutic and/or preventive components, and therefore possess more bioactivity than the synthetic drugs which are often single products. Several *in vitro* or *in vivo* studies have proved the anticancer potential of the herbal extracts[3-5].

Cucurbita pepo (CP), *Cucumis melo* (CM) and *Cucumis sativus* (CS) belong to Cucurbitaceae family, also called as cucurbits, which are consumed as nutritious snacks as they are rich in phytonutrients and have a wide arena of ethnopharmacological relevance. Cucurbit seeds and seed oil are rich in minerals, vitamins, flavonoids, lignans, triterpenes, phytosterols, carotenoids, proteins, tocopherols and polyunsaturated fatty acids, which have shown anti-inflammatory, antiulcer, anthelmintic, antifungal, antibacterial, antiviral, antidiabetic, and antitumor activity[6-10].

In this study, we explored the effect of the seed extracts of *Cucurbita pepo*, *Cucumis melo* and *Cucumis sativus* towards the cytotoxic, antimitotic, and antiproliferative activities using brine shrimp lethality bioassay, *Vigna radiata* germination test, *Allium cepa* root growth inhibition test and yeast model.

Materials and Methods

Samples collection and authentication

Cucurbita pepo (CP), *Cucumis melo* (CM) and *Cucumis sativus* (CS) seeds were obtained from local market, Mumbai, Maharashtra, India. The plants seeds were authenticated by Dr. AS Upadhye, the Agharkar Research Institute, Pune, with voucher specimen no.3/187/2016/Adm-2472/143/144/145, respectively.

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[Suppl. data available in NOPR at http://nopr.niscair.res.in]

Preparation of extracts

The seeds of CP, CM and CS were air dried under the shade and grinded to coarse powder. The powdered seeds (25 g each) were individually extracted by Soxhlet extraction with petroleum ether (60-80°C) / *n*-hexane (350 mL) at 55-60°C temperature for 24-48 h. The extracts were then filtered and concentrated by evaporating the solvents on evaporating dish. The extracts thus obtained were then stored in amber coloured air tight containers in the refrigerator.

Qualitative phytochemical screening

The extracts were subjected to qualitative phyto-chemical screening as described in C.K.Kokate[11] to find the presence of various class of phytoconstituents[11].

Brine shrimp lethality bioassay

The brine shrimp lethality bioassay is a simple, rapid and inexpensive method for testing the cytotoxic potential of plant extracts. Brine shrimps (*Artemia salina*) eggs were purchased online. Brine shrimp eggs were placed in a conical vessel/tank filled with sea water and were allowed to hatch for 48 h under constant light and aeration. After hatching, active nauplii free from the egg shells were then used for testing purpose. Stock solutions of each plant extracts were prepared by dissolving 40 mg of extract in 0.2 mL of DMSO, and finally the volume was made up to 20 mL with sea water. Different concentrations of plant extracts (4000, 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.625 and 7.8125 µg/mL) were then made from this stock solution. The stock of 2.5 Ml of each concentrations was added to 2.5 mL of sea water in different test tubes. Dimethylsulfoxide (DMSO) was used as control. Ten nauplii were introduced into each test tube using a plastic Pasteur pipette. The test tubes were placed uncovered under lamp for 24 h and number of dead nauplii were counted after 24 h. The percentage mortality (%M) was calculated by dividing the number of dead nauplii by the total number and then multiplied by 100%. The lethal concentration (LC_{50}) values were obtained from the best-fit line plotted concentration verses percentage mortality[12,13].

***Allium Cepa* root growth inhibition test**

The antimitotic activity was evaluated using *Allium cepa* roots. *Allium cepa* bulbs were cleaned with water, old roots removed, and The were grown in fresh distilled water for 24 h and then exposed for 4-5 days to different concentrations (250, 500 and 1000 ppm) of each plant extracts and control (distilled water). The bulbs were exposed to fresh test solutions and distilled water every 24 h. After 24 h, the length of the roots for each test solution was measured and the percentage of inhibition of the root growth for each test solution was calculated. The concentration that decreased root growth by 50% when compared to the control group (distilled water) was considered as the efficient concentration (EC_{50}) value. The percentage of inhibition of the root growth was calculated as[14,15]:

$$\text{Percentage of root growth inhibition} = \frac{\text{Root length}_{\text{control}} - \text{Root length}_{\text{extract}}}{\text{Root length}_{\text{control}}} \times 100$$

***Vigna radiata* (Green gram) germination test**

Vigna radiata germination test is a simple, inexpensive and quantitative method for screening herbal extracts for their antimitotic activity. Fourty mg of each plant extract was dissolved in DMSO and then diluted with distilled water. 0.5-1.0 mL of these extract solutions were taken in different test tubes. 1-5 weighed dried green gram seeds were treated with each extract solution along with DMSO and distilled water as the control. The test tubes were covered and left at 37°C for 24 h for imbibition. After 24 h, the seeds were dried using a dry tissue paper and weighed. The percentage of inhibition was calculated as[15,16]:

$$\text{Percentage of inhibition} = \frac{(\text{WtD} - \text{WtE})}{(\text{WtD} - \text{WtDMSO})} \times 100$$

where; WtD is the wet weight of the seed in distilled water; WtE, the wet weight of the seed in experimental sample and WtDMSO, the wet weight of the seed in DMSO.

(Wet weight = weight of seed after imbibition – weight of seed before imbibition)

For further morphological studies, the time of sprouting was extended to 96 h, and photographs were taken.

Antiproliferative assay using yeast (*Saccharomyces cerevisiae*)

Antiproliferative activity of plant extracts was evaluated using yeast (*Saccharomyces cerevisiae*) model. The yeast was inoculated in sterilized nutrient broth and incubated at 37°C for 24 h, and was referred to as "seeded broth". One mL of seeded broth was taken and diluted with sterilized distilled water to

contain 25.4×10^4 cells. Potato dextrose broth was prepared by boiling the un-peeled potato slices in 1.0 L of distilled water for 1 h and was filtered through muslin cloth. The volume of broth was made up to 1.0 L with distilled water and 20 g of dextrose was added to it. Potato dextrose broth was then sterilized by autoclaving. For the cell viability count, 0.5 ml of yeast inoculum and 2.5 mL of potato dextrose broth was treated with 1 mL of each concentration (1, 2, and 3 mg/mL) of each plant extract dissolved in 100% DMSO. It was then incubated at 37°C for 24 h, and the same was done with the control (DMSO). In the cell suspensions thus obtained, 0.1% methylene blue dye was added and the contents were observed under a low power microscope. The number of viable cells (unstained transparent oval cells) and the number of dead cells (stained blue cells) were counted using a haemocytometer. The cells per mL and percentage of the cell viability were calculated using the following formula[17]:

Viable cells / mL = average number of viable cell in one square × dilution factor × 10^4

Percentage of cell viability = Total viable cells / Total cells × 100

Statistical analysis

Each point is the mean of three different replicate experiments, each performed in triplicate. The values are expressed as Mean ± SEM.

Results and Discussion

It has been reported that cucurbit seeds contain compounds like tocopherols, squalene, cucurbitosides, sterols, stigmasterol, polyunsaturated fatty acids and cucurbitacins[8-10]. Phytochemical screening of *n*-hexane and petroleum ether extracts of CP, CM and CS revealed the presence of alkaloids, flavonoids, steroids, carbohydrates, proteins, fats & oils[11]. Seeds of *Cucurbita pepo*, *Cucumis melo* and *Cucumis sativus* have been reported to possess anti-inflammatory, antiulcer, anthelmintic, antifungal, antibacterial, antiviral, hepatoprotective, anti-hypertensive, immunomodulatory, antidiabetic and anticancer effects[6,7].

Brine shrimp (*Artemia salina*) bioassay has been utilized by Meyer *et al*.[13] as a simple, economic and safe pre-screening tool for antitumor drug research and to determine bioactivities of plant products. A crude plant extract is active if it has an LC_{50} value of ≤1000 µg/mL and inactive when it is >1000 µg/mL. The results of brine shrimp lethality bioassay exhibited significant concentration-dependent cytotoxic effect by *n*-hexane and petroleum ether extracts of seeds of CP, CM and CS. The LC_{50} values for *n*-hexane extracts of CP, CM and CS were 750, 250 and 500 µg/mL, respectively while the petroleum ether extracts of CP, CM and CS showed LC_{50} values 500, 500 and 1000 µg/mL, respectively (Fig. 1). The degree of lethality was directly proportional to concentration of extracts.

The antimitotic effects of *n*-hexane and petroleum ether extracts of CP, CM and CS was studied by analyzing the imbibition and sprouting of *Vigna radiata* and by analyzing the root growth of *Allium cepa*. In *Allium cepa* root growth inhibition test, a significant decrease in the root length of the *Allium cepa* bulbs exposed to the extracts was observed as compared to control (distilled water) (Table 1). The degree of root growth inhibition was directly proportional to the concentration of extracts. The average root length of *Allium cepa* bulb in control

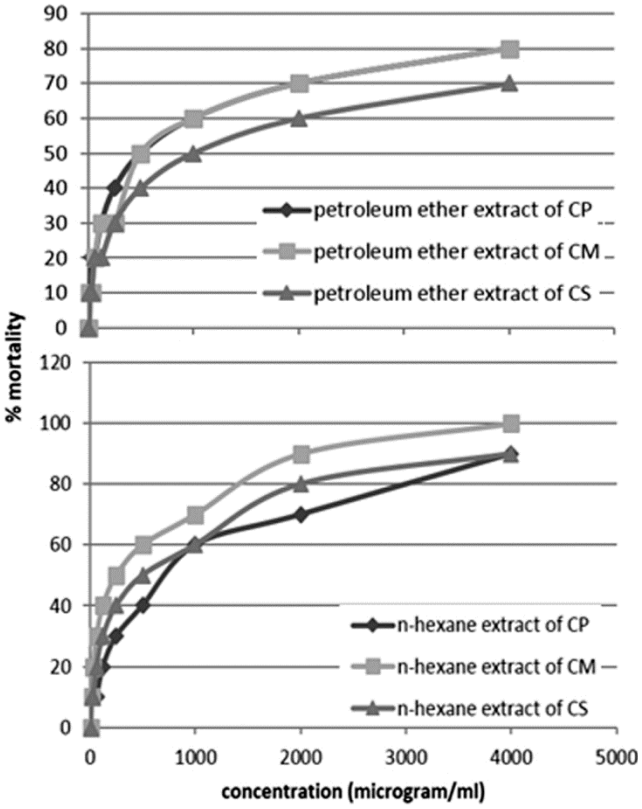


Fig. 1—Percentage mortality of brine shrimp by petroleum ether & n-hexane seed extracts of *Cucurbita pepo* (CP), *Cucumis melo* (CM) and *Cucumis sativus* (CS)

(distilled water) was 11 cm. The EC_{50} values for *n*-hexane and petroleum ether extracts of CP, CM and CS were 500, 500, and 250 ppm, respectively (Fig. 2).

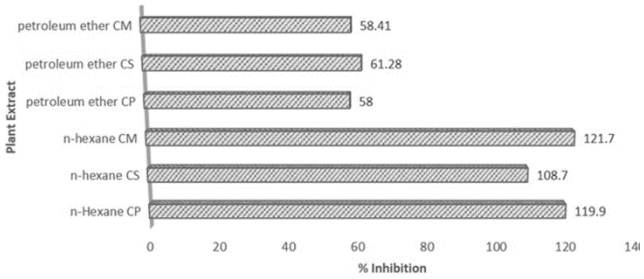
In *Vigna radiata* (green gram) germination test, it was observed that all the extracts were able to inhibit the imbibition mode after 24 h of imbibition stage. Imbibition of seeds was associated with the rupture of seed coat and increase in the seed size. The *n*-hexane extracts showed more inhibition of imbibition mode than the petroleum ether extracts (Fig. 3). Inhibition in the morphology mode was also observed in all the extracts. In the morphology mode, the seeds treated with the extracts for 96 h showed inhibited sprouting, decreased radical length and decreased growth with shortened shoot and small leaves and with no visible roots. While the seeds treated with distilled water and DMSO used as the control showed sprouted seeds had developed roots, shoot and two leaves. The *n*-hexane and petroleum ether extracts of CP, CM and CS caused concentration-dependent inhibition of growth of *Vigna radiata* and inhibition of *Allium cepa* root growth.

In the antiproliferative assay using yeast (*Saccharomyces cerevisiae*) model, all the plant extracts showed good inhibition of yeast cell growth. The degree of cell growth inhibition was directly proportional to concentration of plant extracts [(Table 2 and Suppl. Fig. 1. Suppl. Fig. is available at http://nopr.niscair.res.in)]. The results of the percentage of yeast cell viability clearly showed a concentration-dependent antiproliferative

Table 1—Percentage inhibition of *Allium cepa* root growth by *n*-hexane and petroleum ether extracts of CP, CM and CS

Extract		% Root growth inhibition at different concentrations		
		1000 ppm	500 ppm	250 ppm
n-Hexane	CP	63.6 %	52.7 %	45.4 %
	CM	68.1 %	53.6 %	46.3 %
	CS	81.8 %	62.7 %	52.7 %
Petroleum Ether	CP	68.1 %	50.9 %	40.9 %
	CM	81.8 %	65.4 %	51.8 %
	CS	69 %	56.3 %	47.2 %
Distilled water	Cont.	100 %	80 %	60 %

[CP, *Cucurbita pepo*; CM, *Cucumis melo*; and CS, *Cucumis sativus*]



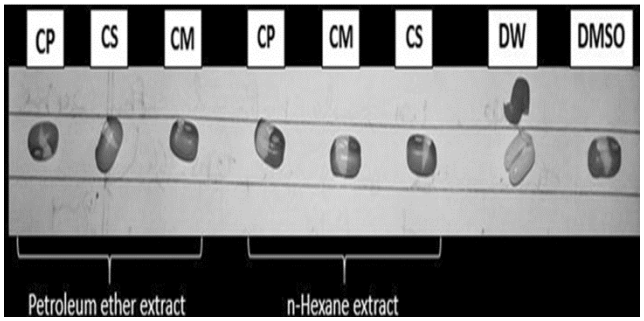


Fig. 3—Percentage of inhibition in *Vigna radiata* germination test by *n*-hexane and petroleum ether extracts of CP, CM, and CS and distilled water

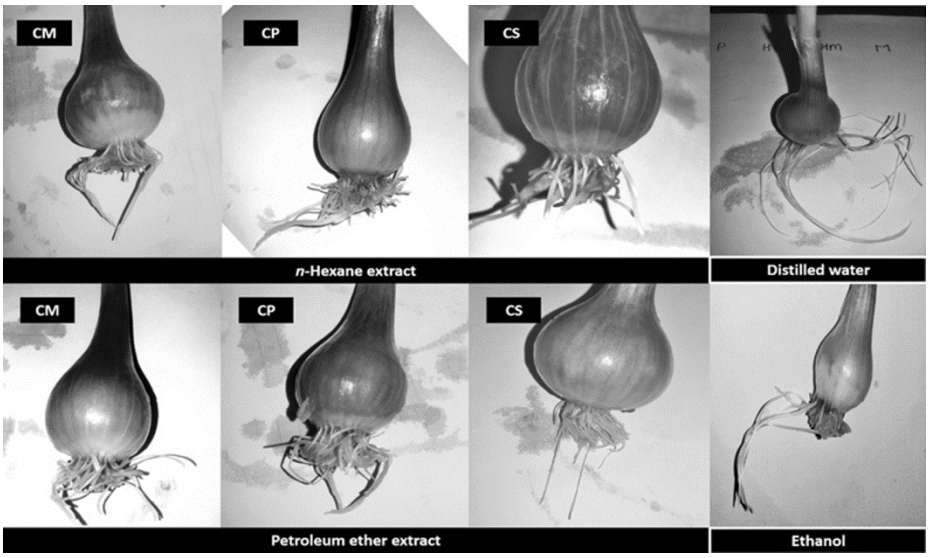


Fig. 2—Effect of *n*-hexane and petroleum ether extracts of CP, CM and CS on *Allium cepa* roots

Table 2—Effect of *n*-hexane and petroleum ether extracts of CP, CM, and CS on the yeast cell viability

Plant extracts		Antiproliferative assay using yeast model								
		% Cell viability			% Cell inhibition			Viable cells/mL		
Concentration (mg/mL)		1	2	3	1	2	3	1	2	3
n-Hexane extract	CP	24.9±0.65	17.2±0.36	4.3±0.10	75	82.7	95.6	177.8×10^4	70.4×10^4	11×10^4
	CM	24.1±0.85	15.6±2.11	2.3±0.43	75.8	84.3	97.6	172.2×10^4	64×10^4	6×10^4
	CS	27±4.0	18.2±1.74	7.4±0.52	72.9	81.7	92.5	193.2×10^4	74.4×10^4	19×10^4
Petroleum ether extract	CP	29.4±0.61	23.5±0.50	9.8±0.61	70.5	76.4	90.1	210×10^4	96×10^4	25×10^4
	CM	27.6±1.21	21.1±1.15	8±0.85	72.3	78.8	91.9	197.4×10^4	86.4×10^4	20.5×10^4
	CS	27.2±1.90	17.8±0.72	6.8±1.12	72.7	82.1	93.1	194.6×10^4	92.8×10^4	17.5×10^4

[CP, *Cucurbita pepo*; CM, *Cucumis melo*; and CS, *Cucumis sativus*]

effect by the *n*-hexane and petroleum ether extracts of CP, CM and CS.

Conclusion

Overall, the results of the current study that explored the cytotoxic, genotoxic, antimitotic, and antiproliferative activity of the crude seed extracts of pumpkin *Cucurbita pepo*, muskmelon *Cucumis melo* and cucumber *Cucumis sativus* have confirmed the above potential. It suggests that, CP, CM, CS seeds have beneficial effects as a medicinal herb and can have a therapeutic potential to destroy cancerous cells.

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