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Evaluation of *In-vitro* Cytotoxicity of *Monochoria vaginalis, Ipomoea carnea, Nardostachys Jatamansi* Extracts on Hela Cells.

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ABSTRACT

The present study was to analyze the anticancer property of *Monochoria Vaginalis, Ipomea Carnia, Nardostachys Jatamansi*on HeLa cells. The Indian medicinal plants used for cancer and noncancerous diseases were collected for the activity. The crude extracts were prepared by using standard protocols. The anti-proliferative effect of plant extracts was evaluated invitro by employing MTT assay. The potency of each plant extract concentration was calculated in terms of percent decrease in viable HeLa cells as compared to the control value. The compounds have shown an *in vitro* cytotoxic effect at different concentrations ranging from 200-1000µg/ml, against human cervical carcinoma (HeLa) cell line. All the extracts have shown reasonable activity at 200µg/ml.

Keywords: HeLa, *Ipomea Carnia, Monochoria Vaginalis,* MTT Assay, *Nardostachys Jatamansi*

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INTRODUCTION

Different types of disease in human beings are cured by plants from many years. As per WHO about 80% of world's problem is treated by medicinal plants. Plant based medicines are the effective source in curing the cancer and the mechanism of action based on phytochemicals also been studied extensively [1, 2]. Various herbal drugs are reported as anticancer agents in Ayurvedic system of medicine. Many clinically useful anticancer drugs are derived from plant origin like vinblastine, vincristine, camptothecin derivatives, topotecan, irinotecan, etoposide derived from epipodophyllotoxin and taxol[3]. A leading mortality worldwide is caused by cancer due to conventional chemotherapy; this shows the critical need of new approaches. The recent research in cancer therapy is to focus on the development of new chemotherapeutic agents from the plant origin. In this study we have investigated the cytotoxic effects of various plants extract like *MonochoriaVaginalis*(*M.Vaginalis*), *IpomeaCarnia*(*I.Carnia*),*NardostachysJatamansi*(*N.Jatamansi*).Efforts are being made to develop safe and cost effective anticancer agents from natural sources.

N. jatamansi is a well-known traditional medicinal plant belonging to family Valerianaceae. The plant has been used for nervous headache, excitement, menopausal symptoms, flatulence, epilepsy, insomnia, disorders of cardiovascular system and intestinal colic for many years traditionally [4, 5]. The extract of *N. jatamansi*arehas anticonvulsant, hepatoprotective and hypolipidemic[6,], antioxidant, lipid peroxidation, fungicidal activity [7].The main active constituents in the plant material aresesquiterpenes and coumarins[8]. Jatamansone or valeranone is the principal sesquiterpene[9]. Other sesquiterpenes include Nardostachone, Dihydrojatamansin, Jatamansinol, Jatamansic Acid [10],jatamansinone, jatamansinol, oroseolol, oroselone, seselin, valeranal, nardostachyin[11](Rucker et al., 1993), nardosinone, spirojatamol[12], jatamol A and B[13], calarenol [14], seychellene, seychelane,coumarin: jatamansin or xanthogalin[15] . More over roots contain valeranone, valeranal, nardone, calarenol, nardostechone,n- hexacosanylarachidate, 8 n-hexaconsanol, calaArene, n- hexacosane, n- hexacosanylisovalerate, ß – sitosterol. norseychelanone, seychellen, patchouli alcohol and ß – patchoulenese[16] , roots oil contains Terpeniccoumarins, oroselol, jatamansin, hydrocarbons, ß - eudesmol, elemol, ß - sitosterol, angelicin, jatamansinol[17].

M.vaginalis commonly belongs to family Pontederiaceae and is distributed throughout India. The leaf juice is used to treat cough and that of roots is used to treat stomach and liver problems, asthma and tooth ache [18, 19]. It is resistant to several acetolactate synthase (ALS) inhibitors (Hwang et al., 2001). The n-butanolfraction of*M. vaginalis*has antioxidant activity[20] and antidiabetic and Hypolipidemic[21] . The ethanol extract of *M. vaginalis* can prevent renal damage from APAP induced nephrotoxicity in rats [22].

I. carnea, glory species with aquatic habitats belongs to the family Convulvulaceae; and distributed throughout India. The plant possess various bioactive compounds such as glycosides, alkaloids, reducing sugars, flavones, fatty acid, esters, and alcohol[23], flavonoids and tannins[24]. Hexadecanoic acid, steric acid, 1, 2 diethyl phthalate, n-octadecanol, octacosane, hexatriacontane, tetraacontane, 3-diethylamino-1-propanol are the active constituents isolated from the leaf extract[25] . The various extracts and isolated compounds of this plant have anti-inflammatory[26],wound-healing activity [27],antioxidant activity [28, 29],antihyperglycemic activity[30]. Most of the synthetic compounds will be having cytotoxic effects towards normal cells. Hence, the focus is on natural products for causing the epigenetic reversal[31]. Because of the safety, relative to cytotoxic synthetic agents the medicinal plants have emerged as attractive candidates for cancer chemoprevention on to the medicinal importance of these plants, present study was undertaken to check these extracts for their ability to inhibit cancer[32].

MATERIAL AND METHODS

Collection and preparation of seed extract of *N. Jatamansi*

The seeds of *N. jatamansi*was collected from Salem district, Tamilnadu and authenticated by Dr. A. Balasubramanian, Siddha research consultant, ABS Botanical garden, Salem, Tamilnadu. The air dried seeds (200g) were powdered and extracted with 50% ethanol in soxhlet apparatus for 72hours. The extract was evaporated under reduced pressure to give solid.

Collection and Preparation leaf extractof *M. vaginalis*

The leaves of *Monochoria vaginali* used for the present study were obtained from Therur pond, Kanyakumari district, Tamil Nadu, India. The whole plant was authenticated by V.Chelladurai, Research officer-Botany (Scientist-c), Central council for Research in Ayurveda & Siddha, Govt. of India. The leaves were collected, shade dried and coarsely powdered by using mechanical grinder. About 200 grams of coarsely powdered leaf material was extracted with 50% ethanol by continuous hot percolation process at 70^{o}C in a Soxhlet apparatus (1000 ml) for 72 hours then it was concentrated by distillation process and evaporated to dryness.

Collection and Preparation whole plant extract of *I. Carneajacq*

The species for the proposed study, *I.carneajacq* leaves were collected in the month of March 2013 from the village Karatupallayalam of Erode district, TamilNadu, India. The species was identification and authenticated as *I. carnea* by Dr.P. Satyanarayana scientist & Head of the office Government of India, Botanical survey of India, Southern Circle, T.N.A.U. Campus, Lawley Road, Coimbatore. A voucherspecimenNo.BSI/SRC/5/23/2011/Tec h-1824 is deposited there. The fresh plant materials are washed and were dried in a shade and ground to powder. About 250gms of dried powdered *I. carnea* leaf was taken in Soxhlet apparatus and extract with measured volume of solvent (80:20 ethanol-water) 72 hours. The temperature was maintained at 55^{o}C- 65^{o}C. Extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness.

Cell culture

HeLa cell line was maintained in DMEM medium (GIBCO) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) and 1% antibiotic solution (penicillin 100Uml^{-1} and streptomycin 100µgml^{-1}) at 37°C in a humidified atmosphere of 95% air/5% CO_2. The medium was changed every second day, and cells were subcultured when confluency reach to 95% by 0.25% trypsin containing 0.02% ethylene-diaminetetra acetic acid (EDTA) in PBS for 3 min at 37°C.

MTT Assay

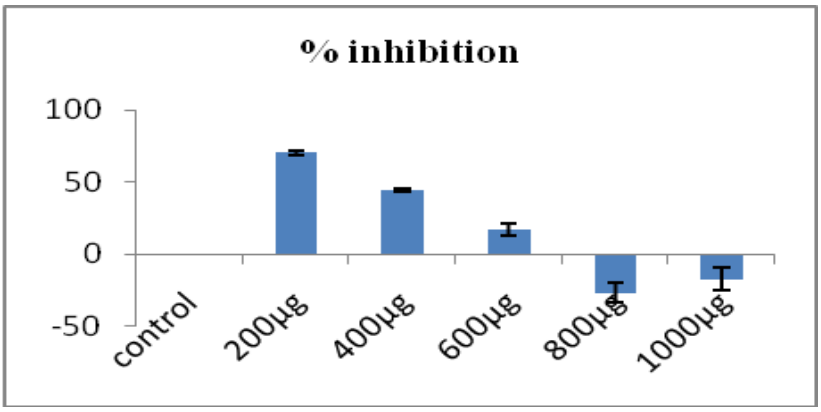
The MTT assay was carried out as described previously to measure cell viability [33]. Ten thousand cells in 100µL of DMEM media were seeded in the wells of a 96-well plate. After24 h, existing media was removed and 100 µL of various concentrations of extracts was added and incubated for 48 h at 37°C in a CO_2 incubator. Control cells were supplemented with 0.05% DMSO vehicle. At the 48th hour of incubation, MTT (3-(4,5-dimethylthaizol-2-yl)-2,5-diphenyltetrazolium bromide- supplied from Sigma, 10µL of 5 mg/mL) was added to the plate. The contents of the plate were pipetted out carefully, the formazan crystals formed were dissolved in 100 µL of DMSO, and the absorbance was measured at 550 nm in a microplate reader (Tecan, infinite F200 Pro). Experiments were performed in triplicate, and the results were expressed as mean of percentage inhibition. A graph of the concentration versus percentage growth inhibition was plotted, and the concentration at which 50% cell death occurred was considered as the IC50 value. Before adding MTT, bright field images (Olympus 1X81, cellSens Dimension software) were taken for visualizing the cell death.

RESULTS AND DISCUSSION

The result reveals the percentage yield for theseeds of *N. jatamansi* extracted with 50% ethanol to be 8.2%w/w, for *M. vaginalis*leaf extracted with 50% ethanol to be 5.8% w/w and for the *I .carnea* leaf extract with measured volume of solvent (80:20 ethanol-water) to be 4.7 % w/w. The present experiment to analyze the anticancer property of *Monochoria Vaginalis, Ipomea Carnia, Nardostachys Jatamansi*on HeLa cells obtained moderate results with the treatment of these extracts morphological changes in the cells were observed. *M. Vaginali s*and *N. Jatamansi* extracts has obtained IC_{50} value at 200 µg/ml (figure 1&3). Whereas *I. Carnia* extract failed to exhibit IC_{50} value, even though it has showed minimal anticancer activity (figure 2).Figure 4 depicts the cytotoxic effect to extracts on HeLa cell line. Moreover; the plant extracts contain natural compounds which do not have any cytotoxic effects on normal cells unlike the demethylating chemicals [31]. In earlier studies, HPTLC analysis of the fractions of *N. jatamansi* confirmed the presence of lupeol and β-sitosterol, these compounds have the ability to inhibit the proliferation of MCF-7, MDA-MB-231,

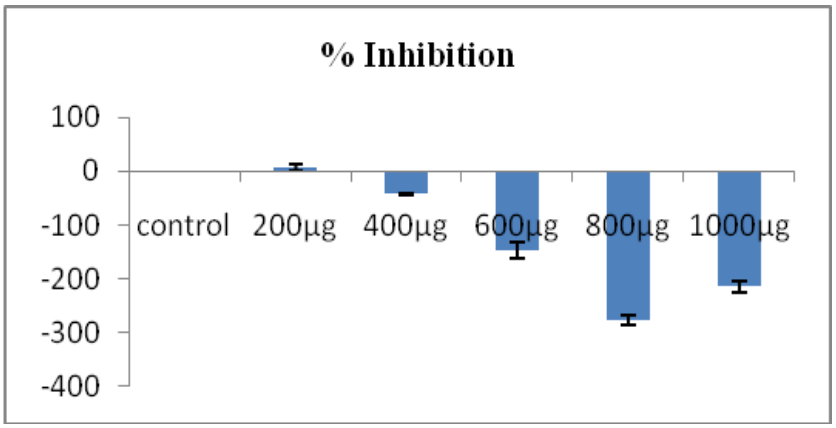
and other breast cancer cells[34, 35]. The terpenoids(6R,9S)-vomifoliol, (6S,9S)-vomifoliolwhich are present in the extract of *Monochoria vaginalis* probably due to cause for the activity [36].

Figure1: Percentage inhibition of cell growth at different concentrations of *Monochoria Vaginalis extracts*



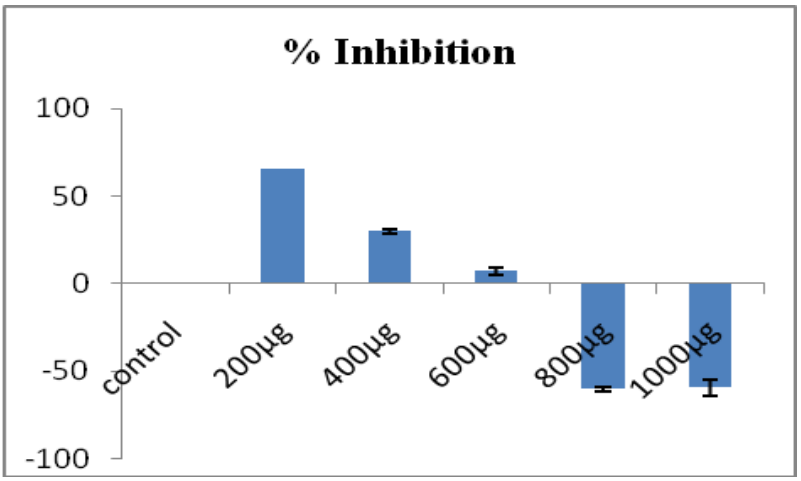
Data are Mean±SE (n=3)

Figure 2: Percentage inhibition of cell growth at different concentrations of *Ipomea carnea* extracts



Data are Mean±SE (n=3)

Figure 3: Percentage inhibition of cell growth at different concentrations of *Nardostachys Jatamansi* extracts.



Data are Mean±SE (n=3)

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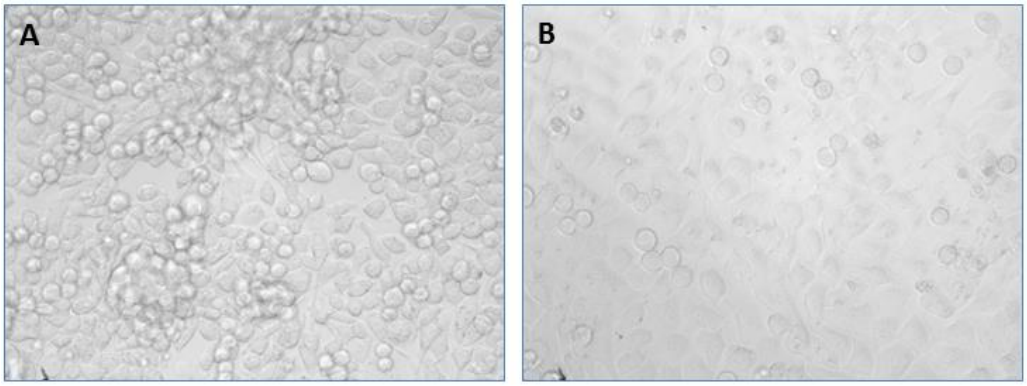
Figure 4: Anticancer activity of extracts showing cell death, A-control; B-treated.



The present study could prove to be an important step in the direction of therapy against cancer. *M. Vaginalis*and *N. Jatamansi*extractsproved to be effective amongst all the three plant extracts. However, it is necessary to perform many other studies both *in vitro* and *in vivo* to determine their true potential for the development of anticancer drugs.

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