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Study on Medicinal Plants of Kashmir Valley for Anti-Proliferative, Anti-Invasive Activities against Prostate Cancer

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A B S T R A C T

Treatment of castration-resistant prostate cancer (CRPC) patients with androgen deprivation therapy puts prostate cancer in remission while treatment with already available drugs in market including abiraterone help in controlling advanced prostate cancers for sometime though fail to respond, evolve resistance mechanisms, and undergo genetic deregulations later on with poor patient survival rate and no cure. Also, if present trends of increasing life expectancy continue, given the current age-specific incidence, mortality rates of prostate cancer, this disease will become a far greater health problem worldwide in future. For this reason, addressing the curative treatment strategies for prostate cancer was the focal theme of our investigation. Our emphasis was on the extracts from medicinal plants of Kashmir Valley, which we collected from different floristically rich regions of Valley including Leh-Ladakh, Gurez, Dachigam National Sanctuary, Jawahar Lal Nehru Memorial Botanical Garden, Medicinal Plants Emporium Srinagar, Faculty of Forestry, SKUAST-K, Kangan and local nurseries in Srinagar area. In this study, we screened library of 372 extracts from collected medicinal plants (52) for their antiproliferative and anti-invasive efficacy through colony forming units and wound healing assays, which led to the identification of leaf extract of *Podophyllum hexandrum* as inhibitor molecule.Wound healing assay revealed that in presence of this extract, cancer cells show inhibition of cell migration, thus showing detrimental effect on invasiveness of C4-2 cells. CFU assay depicted inhibition of cellular proliferation and reduced colony forming units in C4-2, LnCaP and PC3 cells with increasing concentrations of this extract. Potential of this extract as a lead compound for the development of new treatment options for CRPC, including those resistant to enzalutamide, abiraterone and other anti-androgens could be explored through future studies. Also, different extracts of this plant will act as tool for evaluation of wide range of biological activities.

Introduction

Utilization of plants for medicinal purposes dates back to centuries, even before long recorded history(Jamshidi-Kia, Lorigooini *et al.,* 2018). Primitive men valued, appreciated the great diversity and helpfulness of plants accessible to them(Li and Lou 2018). As times passed by, each tribe added the medicinal power of herbs in their field to its knowledge base(Dereli, Ilhan *et al.,* 2019).Treatment of number of diseases including diabetes with plant-derived drugs are the earliest success stories(Jacob, Li *et al.,* 2019). Today, we are more concerned with the life-style diseases like cancer (Zhong, Pascal *et al.,* 2018). With cancer being a boundless risk to the mankind, plants in the form of useful products can assume a significant job in cancer counteractive action, as well as in its therapy(Jing, Nguyen *et al.,* 2018). Globally cancer is a disease that seriously effects the human population and as a consequence there is a consistent interest for development of new treatments to prevent this perilous disease(Masoodi, Xu *et al.,* 2017). Scientific and research intrigue is constantly drawing its consideration towards naturally-derived compounds(Isgut, Rao *et al.,* 2018) as they are considered to have less toxic side effects contrasted with current modes of treatment using allopathy as well as chemical induced processes such as chemotherapy (Seca and Pinto 2018). Plants therefore, have been indispensable in treating diverse forms of diseases including cancer (Buyel 2018).

In recent years, medicinal plants have occupied an important position in being the paramount sources of drug discovery, irrespective of their categorized groups- herb, shrub or tree (Tewari, Rawat *et al.,* 2019). These practices have solely been based on the knowledge of traditional use of medicinal plants(Kaushik and Kaushik 2018). Proper understanding of the complex synergistic interaction of various constituents of anticancer herbs(Agyare, Spiegler *et al.,* 2018), should therefore, help in formulating the treatment design to attack the cancerous cells without harming the normal cells of the body(Bhat, Gul *et al.,* 2018).The Plant kingdom produces naturally occurring secondary metabolites that are being investigated for their anticancer activities leading to the development of new clinical drugs(Ullah and Ahmad 2019). With the success of these compounds that have been developed into staple drugs for cancer treatment, new technologies are emerging to develop the area further(Rupani and Chavez 2018). Thus, there is lately a great deal of interest in screening plants to be eventually used in cancer prevention and treatment.

Among different types of cancers, prostate cancer is one of the chief reasons for mortalities in men worldwide(1,276,106 number of new cases [7.1% of total cases of cancer], 358,989 number of death [3.8% of total cancer deaths]as of 2018)(Keavey and Thompson 2018)and medical castration is the standard-care treatment for the patients (Aw-Yong, Gan *et al.,* 2018). Aggressive prostate cancers have a progressive and morbid disease process with a median survival of 9-30 months(Johnston, Nguyen *et al.,* 2016). Androgen-deprivation therapy puts prostate cancer in remission(Eisermann, Dar *et al.,* 2015), whereas hormonal therapies help in controlling advanced prostate cancers for sometime though fail to respond (Khan and Gurav 2018), evolve resistance mechanisms, and undergo genetic deregulations later on with poor patient survival rate and no cure(Wang, Nguyen *et al.,* 2015). If the present trends of increasing life expectancy continue, given the current age-specific incidence, morbidity and mortality rates of prostate cancer(Kebebe, Liu *et al.,* 2018), this disease will become a far greater health problem worldwide in future(Pascal, Masoodi *et al.,* 2015).

For this reason, addressing the curative treatment strategies of prostate cancer was the focal theme of our investigation. And for the same, we screened library of 372 plant extracts for their capability to inhibit colony forming units and cell migration/movement of castration resistant prostate cancer cells, which led to the identification of leaf extract of *Podophyllum hexandrum* as inhibitory extract in both the assays.

Materials and Methods

Plant material

52 medicinal plants both fresh as well as dried (leaves, roots, flowers, seeds, fruit, bark and other parts) were collected in different seasons (flowering as well as fruiting), from different regions of Kashmir Valley (including Leh-Ladakh, Gurez, Jawahar Lal Nehru Memorial Botanical Garden Srinagar, Medicinal Plants Emporium Srinagar, Faculty of Forestry, Benihama) during 2016-2017 (Figure 1a-1e). Voucher specimens were deposited at the SKUAST-K herbarium. Fresh plant parts were washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in airtight bottles/ ziplocks. Dried plant parts were also subjected to grinding and stored in airtight ziplocks. Plants used for investigation of anticancer activity were subjected to following methods:

Preparation of extracts

For preparation of extracts, 20-25gms of powder of each plant part was subjected to soxhlet extraction with different solvents for 48 hrs. For each plant part, six extracts were obtained. The extracts were dried using rotary vacuum evaporator, dissolved in 10ml DMSO, filter sterilized, weighed and stored in -20^{o}C until tested. The extracts were formatted at 12.5mg/ml concentration, from which further dilutions were made in RPMI-1640 medium at the time of testing.

Reagents

Dimethyl sulfoxide (DMSO), phosphate-buffered saline (PBS) (Cat no. TL1031), Cyclohexane, Hexane, Diethyl ether, Ethyl acetate, Methanol were obtained from Himedia. RPMI-1640 medium (Cat no.11875-093), L-glutamine (Cat no.25030081) ,fetal bovine serum (FBS), trypsin (Cat no. 25200056) were obtained from Gibco/Life Technology .

Cell culture establishment

C4-2 cells were obtained from Zhou Wang's Laboratory, University of Pittsburgh, under MTA with MD Anderson Cancer Centre Texas USA and LnCaP, and PC3 cells were obtained from NCCS Pune. Cell lines were maintained in the RPMI-1640 medium supplemented with 10% FBS, 1% Pennstrep and 1% L-glutamine at 37^{o}C with 5% CO_2 (Figure 2).

Colony forming Unit Assay (CFU)

The colony forming unit assay was utilized to determine the effect of extract library on the cell regrowth of prostate cancer cells.

All the three cell lines (C4-2, LnCap, PC3) were seeded in 12 well plates prior to treatment with extracts. Cell lines at 70-90% confluency was washed with PBS and treated with different concentrations of plant extracts (3.12, 6.25, 12.5, 25 and 50µg/ml), DMSO control and incubated for 48 hrs. Then an equal number of cells from treated wells were seeded in 10 cm dishes to form colonies for atleast 7 to 10 days. After removing the media gently from the dishes, sufficient 100% methanol to cover the cells completely was added to plates, which were then incubated for 20 mins. The methanol was removed and cells rinsed carefully with water. Sufficient crystal violet solution was added to cover the cells and incubated for 5 mins. at room

temperature. The cells were washed with water to remove excess dye. Then the plates were kept inverted on tissue paper to dry overnight. The colonies were counted using ImageJ software. Data was analysed using Graph Pad Prism 7.0.

Cell Migration Assay using Wound Healing

C4-2 cells were grown in 24-well plates to 70-80% confluency. Nextday, media was removed from plates and the monolayer was gently scratched with a sterile 200 µl pipette tip across the center of the well (straight scratches were made). While scratching across the surface of the well, care was taken to keep the long-axial of the tip perpendicular to the bottom of the well. After scratching, wells were washed with PBS to remove the detached cells and the wells were filled with fresh RPMI media supplemented with 10% FBS, 1% L-glutamine, 1% Pennstrep. Images of the cellular gap were captured in bright field on LMI inverted microscope before the addition of plant extracts. The cells were then treated with plant extracts at a concentration of 50µg/ml and wells treated with vehicle DMSO was used as control. After addition of plant extracts, the well plates were incubated at 5% CO_2 and 37°C, while capturing the images of the cellular gap periodically at 0hrs and 48 hr timings in bright field on LMI inverted microscope to note down the changes in the gap distance of scratch. The cellular gap distance was quantitatively evaluated as percentage wound area using Graph pad Prism 7.0

Statistical analysis

For statistical analysis and graphical composition, GraphPad Prism 7.0 (GraphPad Software, Inc) and MS Excel 2003 (Microsoft) were used. Data was expressed as the mean ± SEM and to determine statistical significance, one-way ANOVA or Student's t-test was used.

Results and Discussion

Library construction of the extracts from the collected medicinal plants

Crude extract library comprising 372 plant extracts was formed during the investigation (Table 1).We gave our own set of nomenclature to constructed library, according to which the first part of the drug name i.e. 1,2,3,and so represents the sequential location/position of extract in the library. The second part of the drug name is the first letter of the plant part/organ name, that is used in the extract preparation, followed by the third part of the drug name including the first two letters (first letter of generic name and first letter of species name) of the botanical name of the source plant. The last part of the name represents the solvent system, used for extract preparation. Different parts of the drug name are separated from each other by a hyphen. The crude extracts of this library, both serving as drugs and as templates for the synthesis of drugs will serve many researchers and drug companies to facilitate drug discovery as a tool for the evaluation of a wide range of biological activities.

Colony Forming Unit Assay

Podophyllum hexandrum leaf extract inhibited colony forming unit ability of all three cell lines with increasing concentrations (Figure 3).At lower concentrations, there was reduction in number of colonies when compared to control while at higher concentrations the plates were empty with no colonies suggesting the inhibition of colony forming units takes place in a concentration dependent manner (Figure 3).

Cell migration using wound healing assay

Podophyllum hexandrum leaf extract signifi-

cantly inhibited cancer cell migration in time-dependent manner (Figure4). Extract inhibited C4-2 cancer cell migration by 70% at a concentration of 50 µg/ml, in comparison with the untreated control group (Figure 4).There was no significant gap closure in wells treated with *Podophyllum hexandrum* leaf extract while there was near 40% gap closure in control plate.

Table.1 Library construction of the isolated extracts from collected medicinal plants

S. No.	Common Name	Medicinal Plant	Place of collection	Part Used	Nomenclature
01	*Evergreen maidenhair/ Geotheer*	*Adiantum venustum*	Botanical Garden Nursery	Leaves	1- L-AV-CH
					2- L-AV-Hx
					3- L-AV-DE
					4- L-AV-EA
					5- L-AV-Ml
					6- L-AV-Wtr
02	*Bugle weed/ Jaan-e-Adam*	*Ajuga bracteosa*	Faculty of Forestry, SKUAST-K	Leaves	7- L-AB-CH
					8- L-AB-Hx
					9- L-AB-DE
					10- L-AB-EA
					11- L-AB-Ml
					12- L-AB-Wtr
03	*Himalayan arnebia/ Kahzabaan*	*Arnebia enthami*	Faculty of Forestry, SKUAST-K	Tuber	13- T-AB-CH
					14- T-AB-Hx
					15- T-AB-DE
					16- T-AB-EA
					17- T-AB-Ml
					18- T-AB-Wtr
04	*Wormwood / Tethwan*	*Artemisia absinthium*	Botanical Garden Nursery	Leaves	19- L-AA-CH
					20- L-AA-Hx
					21- L-AA-DE
					22- L-AA-EA
					23- L-AA-Ml
					24- L-AA-Wtr
05	*Wormwood / Tethwan*	*Artemisia annua*	Botanical Garden Nursery	Leaves	25- L-AM-CH
					26- L-AM-Hx
					27- L-AM-DE
					28- L-AM-EA
					29- L-AM-Ml
					30- L-AM-Wtr
06	*Zakhmi Hayat*	*Bergenia ligulata*	SKUAST-K,Shalimar	Leaves	31- L-BL-CH
					32- L-BL-Hx
					33- L-BL-DE
					34- L-BL-EA

					35-	L-BL-Ml
					36-	L-BL-Wtr
07	*Bhang*	*Cannabis sativa*	Buchpora, Srinagar	Leaves	37-	L-CS-CH
					38-	L-CS-Hx
					39-	L-CS-DE
					40-	L-CS-EA
					41-	L-CS-Ml
					42-	L-CS-Wtr
08	*Bahuvar*	*Cordia latifolia*	Faculty of Forestry, SKUAST-K	Seeds	43-	S-CL-CH
					44-	S-CL-Hx
					45-	S-CL-DE
					46-	S-CL-EA
					47-	S-CL-Ml
					48-	S-CL-Wtr
09	*Kesar*	*Crocus sativus*	Frestabal, Pampore	Stigma	49-	S-CS-CH
					50-	S-CS-Hx
					51-	S-CS-DE
					52-	S-CS-EA
					53-	S-CS-Ml
					54-	S-CS-Wtr
10	*Haldi*	*Curcuma longa*	Sanatnagar,Srinagar	Rhizomes	55-	R-CL-CH
					56-	R-CL-Hx
					57-	R-CL-DE
					58-	R-CL-EA
					59-	R-CL-Ml
					60-	R-CL-Wtr
11	*Datur*	*Datura innoxia*	Botanical Garden Nursery	Leaves	61-	L-DS-CH
					62-	L-DS-Hx
					63-	L-DS-DE
					64-	L-DS-EA
					65-	L-DS-Ml
					66-	L-DS-Wtr
12	*Yam/Singli Mingli*	*Dioscorea deltoidea*	Faculty of Forestry, SKUAST-K	Rhizome/Leaves	67-	R-BL-CH
					68-	R-BL-Hx
					69-	R-BL-DE
					70-	R-BL-EA
					71-	R-BL-Ml
					72-	R-BL-Wtr
					73-	L-BL-CH
					74-	L-BL-Hx
					75-	L-BL-DE
					76-	L-BL-EA
					77-	L-BL-Ml
					78-	L-BL-Wtr

13	*Yam*	*Dioscorea balcanica*	Faculty of Forestry, SKUAST-K	Rhizome	79- R-DB-CH
					80- R-DB-Hx
					81- R-DB-DE
					82- R-DB-EA
					83- R-DB-Ml
					84- R-DB-Wtr
14	*Foxglove*	*Digitalis lanata*	Botanical Garden Nursery	Seed	85- S-DL-CH
					86- S-DL-Hx
					87- S-DL-DE
					88- S-DL-EA
					89- S-DL-Ml
					90- S-DL-Wtr
15	*Himalayan teasel/ Wopalhaakh*	*Dipsacus inermis*	Faculty of Forestry, SKUAST-K	Leaves	91- L-DI-CH
					92- L-DI-Hx
					93- L-DI-DE
					94- L-DI-EA
					95- L-DI-Ml
					96- L-DI-Wtr
16	*Wopal Haakh*	*Dipsacus mitis*	Dachigam National Sanctuary	Leaves	97- L-DM-CH
					98- L-DM-Hx
					99- L-DM-DE
					100- L - DM-EA
					101- L-DM-Ml
					102- L-DM-Wtr
17	*Maidenhair tree*	*Gingko biloba*	SKUAST-K., Shalimar	Leaves	103- L-GB-CH
					104- L-GB-Hx
					105- L-GB-DE
					106- L-GB-EA
					107- L-GB-Ml
					108- L-GB-Wtr
18	*Mulethi*	*Glycyrrhiza glabra*	Brakpora, Anantnag	Rhizome	109- R-GG-CH
					110- R-GG-Hx
					111- R-GG-DE
					112- R-GG-EA
					113- R-GG-Ml
					114- R-GG-Wtr
19	*Fig*	*Ficus carica*	Buchpora, Srinagar	Fruit	115- F-FC-CH
					116- F-FC-Hx
					117- F-FC-DE
					118- F-FC-EA
					119- F-FC-Ml
					120- F-FC-Wtr
20	*China rose*	*Hibiscus rosa-*	SKIMS Soura,	Petals	121- P-HR-CH
					122- P-HR-Hx

		sinensis	Srinagar		123- P-HR-DE
					124- P-HR-EA
					125- P-HR-Ml
					126- P-HR-Wtr
21	*Seabuckthorn/ Badriphal*	*Hippophae rhamnoides*	Ladakh	Leaves	127- L-HR-CH
					128- L-HR-Hx
					129- L-HR-DE
					130- L-HR-EA
					131- L-HR-Ml
					132- L-HR-Wtr
22	*Bazar bang / Ajwain*	*Hyosyamus niger*	Gawkadal, Srinagar	Seeds	133- S-HN-CH
					134- S-HN-Hx
					135- S-HN-DE
					136- S-HN-EA
					137- S-HN-Ml
					138- S-HN-Wtr
23	*Bottlegourd*	*Lagenaria siceraria*	Buchpora, Srinagar	Seeds	139- S-LS-CH
					140- S-LS-Hx
					141- S-LS-DE
					142- S-LS-EA
					143- S-LS-Ml
					144- S-LS-Wtr
24	*Poppy*	*Papaver somniferum*	SKUAST-K, Wadura	Flower/Leaves	145- F-PS-CH
					146- F-PS-Hx
					147- F-PS-DE
					148- F-PS-EA
					149- F-PS-Ml
					150- F-PS-Wtr
					151- L-PS-CH
					152- L-PS-Hx
					153- L-PS-DE
					154- L-PS-EA
					155- L-PS-Ml
					156- L-PS-Wtr
25	*Mint/ Pudina*	*Mentha longifolia*	Buchpora, Srinagar	Leaves	157- L-ML-CH
					158- L-ML-Hx
					159- L-ML-DE
					160- L-ML-EA
					161- L-ML-Ml
					162- L-ML-Wtr
26	*Mint/ Pudina*	*Mentha arvensis*	Botanical Garden Nursery	Leaves	163- L-MA-CH
					164- L-MA-Hx
					165- L-MA-DE
					166- L-MA-EA

					167- L-MA-Ml
					168- L-MA-Wtr
27	*Lemonbalm*	*Melissa offiinalis*	Botanical Garden Nursery	Leaves	169- L-MO-CH
					170- L-MO-Hx
					171- L-MO-DE
					172- L-MO-EA
					173- L-MO-Ml
					174- L-MO-Wtr
28	*Morchella*	*Morchella esculenta*	Faculty of Forestry, SKUAST-K	Fruit	175- F-ME-CH
					176- F-ME-Hx
					177- F-ME-DE
					178- F-ME-EA
					179- F-ME-Ml
					180- F-ME-Wtr
29	*Mulberry/ Tul*	*Morus alba*	SKUAST-K, Shalimar	Fruit	181- F-MA-CH
					182- F-MA-Hx
					183- F-MA-DE
					184- F-MA-EA
					185- F-MA-Ml
					186- F-MA-Wtr
30	*Buttonweed/ Sochal*	*Malva sylvestris*	Buchpora, Srinagar	Leaves/Flowers	187- L-MS-CH
					188- L-MS-Hx
					189- L-MS-DE
					190- L-MS-EA
					191- L-MS-Ml
					192- L-MS-Wtr
					193- F-MS-CH
					194- F-MS-Hx
					195- F-MS-DE
					196- F-MS-EA
					197- F-MS-Ml
					198- F-MS-Wtr
31	*Oregano/ Marzanjosh*	*Origanum vulgare*	Botanical Garden Nursery	Leaves	199- L-OV-CH
					200- L-OV-Hx
					201- L-OV-DE
					202- L-OV-EA
					203- L-OV-Ml
					204- L-OV-Wtr
32	*Picroliv/ Kutki*	*Picrorhiza kurroa*	Botanical Garden Nursery	Leaves	205- L-PK-CH
					206- L-PK-Hx
					207- L-PK-DE
					208- L-PK-EA
					209- L-PK-Ml

					210- L-PK-Wtr
33	*Himalayan mayapple/ Bankakri/ Wanwangun*	*Podophyllum hexandrum*	Gurez	Fruit/Leaves /Root	211- L-PH-CH 212- L-PH-Hx 213- L-PH-DE 214- L-PH-EA 215- L-PH-Ml 216- L-PH-Wtr 217- F-PH-CH 218- F-PH-Hx 219- F-PH-DE 220- F-PH-EA 221- F-PH-Ml 222- F-PH-Wtr 223- R-PH-CH 224- R-PH-Hx 225- R-PH-DE 226- R-PH-EA 227- R-PH-Ml 228- R-PH-Wtr
34	*Self-heal / Kalaveoth*	*Prunella vulgaris*	Faculty of Forestry, SKUAST-K	Leaves	229- L-PV-CH 230- L-PV-Hx 231- L-PV-DE 232- L-PV-EA 233- L-PV-Ml 234- L-PV-Wtr
35	*Apricot*	*Prunus armeniaca*	Leh, Ladakh	Fruit/ Leaves	235- L-PA-CH 236- L-PA-Hx 237- L-PA-DE 238- L-PA-EA 239- L-PA-Ml 240- L-PA-Wtr 241- F-PA-CH 242- F-PA-Hx 243- F-PA-DE 244- F-PA-EA 245- F-PA-Ml 246- F-PA-Wtr
36	*Pambchalan*	*Rheum emodi*	Faculty of Forestry, SKUAST-K	Leaves	247- L-RE-CH 248- L-RE-Hx 249- L-RE-DE 250- L-RE-EA 251- L-RE-Ml 252- L-RE-Wtr
37	*Rose/ Gulab*	*Rosa damascene*	Dachigam	Petals/	253- P-RD-CH

			National Sanctuary	Leaves	254- P-RD-Hx
					255- P-RD-DE
					256- P-RD-EA
					257- P-RD-Ml
					258- P-RD-Wtr
					259- L-RD-CH
					260- L-RD-Hx
					261- L-RD-DE
					262- L-RD-EA
					263- L-RD-Ml
					264- L-RD-Wtr
38	*Rose/ Gulab*	*Rosa webbiana*	Dachigam National Sanctuary	Petals	265- P-RW-CH
					266- P-RW-Hx
					267- P-RW-DE
					268- P-RW-EA
					269- P-RW-Ml
					270- P-RW-Wtr
39	*Rosemary*	*Rosmarinus officinalis*	Botanical Garden Nursery	Leaves	271- L-RO-CH
					272- L-RO-Hx
					273- L-RO-DE
					274- L-RO-EA
					275- L-RO-Ml
					276- L-RO-Wtr
40	*Toothed dock/ Obej*	*Rumex dentatus*	Faculty of Forestry, SKUAST-K	Leaves	277- L-RD-CH
					278- L-RD-Hx
					279- L-RD-DE
					280- L-RD-EA
					281- L-RD-Ml
					282- L-RD-Wtr
41	*Herb of grace/ Barge/ Burg*	*Ruta graveolens*	Faculty of Forestry, SKUAST-K	Seeds	283- S-RG-CH
					284- S-RG-Hx
					285- S-RG-DE
					286- S-RG-EA
					287- S-RG-Ml
					288- S-RG-Wtr
42	*Santolina*	*Santolina chamaecyparissus*	Botanical Garden Nursery	Flower	289- F-SC-CH
					290- F-SC-Hx
					291- F-SC-DE
					292- F-SC-EA
					293- F-SC-Ml
					294- F-SC-Wtr
43	*Kuth*	*Saussurea lappa*	Botanical Garden Nursery	Leaves	295- L-SL-CH
					296- L-SL-Hx
					297- L-SL-DE

					298- L-SL-EA
					299- L-SL-Ml
					300- L-SL-Wtr
44	*Kuth*	*Saussurea costus*	Botanical Garden Nursery	Root	301- R-SC-CH
					302- R-SC-Hx
					303- R-SC-DE
					304- R-SC-EA
					305- R-SC-Ml
					306- R-SC-Wtr
45	*Clove*	*Syzygium aromaticum*	Soura, Srinagar	Flower buds	307- F-SA-CH
					308- F-SA-Hx
					309- F-SA-DE
					310- F-SA-EA
					311- F-SA-Ml
					312- F-SA-Wtr
46	*Handh/ Dandelion*	*Taraxacum officinale*	Buchpora, Srinagar	Leaves/Root /Flowers	313- L-TO-CH
					314- L-TO-Hx
					315- L-TO-DE
					316- L-TO-EA
					317- L-TO-Ml
					318- L-TO-Wtr
					319- R-RD-CH
					320- R-RD-Hx
					321- R-RD-DE
					322- R-RD-EA
					323- R-RD-Ml
					324- R-RD-Wtr
					325- F-RD-CH
					326- F-RD-Hx
					327- F-RD-DE
					328- F-RD-EA
					329- F-RD-Ml
					330- F-RD-Wtr
47	*Yew/ Postul*	*Taxus buccata*	SKUAST-K, Shalimar	Leaves/Bran ch	331- L-TB-CH
					332- L-TB-Hx
					333- L-TB-DE
					334- L-TB-EA
					335- L-TB-Ml
					336- L-TB-Wtr
					337- B-TB-CH
					338- B-TB-Hx
					339- B-TB-DE
					340- B-TB-EA
					341- B-TB-Ml

					342- B-TB-Wtr
48	*Soi*	*Urtica dioca*	Buchpora, Srinagar	Leaves	343- L-UD-CH
					344- L-UD-Hx
					345- L-UD-DE
					346- L-UD-EA
					347- L-UD-Ml
					348- L-UD-Wtr
49	*Banafsha*	*Viola odorata*	Faculty of Forestry, SKUAST-K	Leaves	349- L-VO-CH
					350- L-VO-Hx
					351- L-VO-DE
					352- L-VO-EA
					353- L-VO-Ml
					354- L-VO-Wtr
50	*Banafsha*	*Viola biflora*	Faculty of Forestry, SKUAST-K	Leaves	355- L-VB-CH
					356- L-VB-Hx
					357- L-VB-DE
					358- L-VB-EA
					359- L-VB-Ml
					360- L-VB-Wtr
51	*Ginger*	*Zingiber officinale*	Buchpora, Srinagar	Rhizome	361- R-ZO-CH
					362- R-ZO-Hx
					363- R-ZO-DE
					364- R-ZO-EA
					365- R-ZO-Ml
					366- R-ZO-Wtr
52	*Shatavari/ Parglas*	*Asparagus racemosus*	Botanical Garden Nursery	Roots	367- R-AR-CH
					368- R-AR-Hx
					369- R-AR-DE
					370- R-AR-EA
					371- R-AR-Ml
					372- R-AR-Wtr

Figure.1a Medicinal Plants collected from different regions of Kashmir Valley

Figure.1b

Medicinal Plants collected from different regions of Kashmir Valley

Asparagus racemosus

Viola purpurea

Origanum vulgare

Viola odorata

Aesculus indica

Rheum emodi

Prunella vulgaris

Taraxacum officinale

Cannabis sativa

Ginkgo biloba

Morchella esculenta

Podophyllum hexandrum

Figure.1c Medicinal Plants collected from different regions of Kashmir Valley

Figure.1d Medicinal Plants collected from different regions of Kashmir Valley

Figure.1e Medicinal Plants collected from different regions of Kashmir Valley

Figure.2 Establishment of Prostate cancer cell lines in RPMI-1640media supplemented with 10% FBS, 1% L-Glutamine, 1% PenStrep and maintained in 5%CO_2 incubator at 37^0C. Representative bright field images at 10X, and 40X magnifications

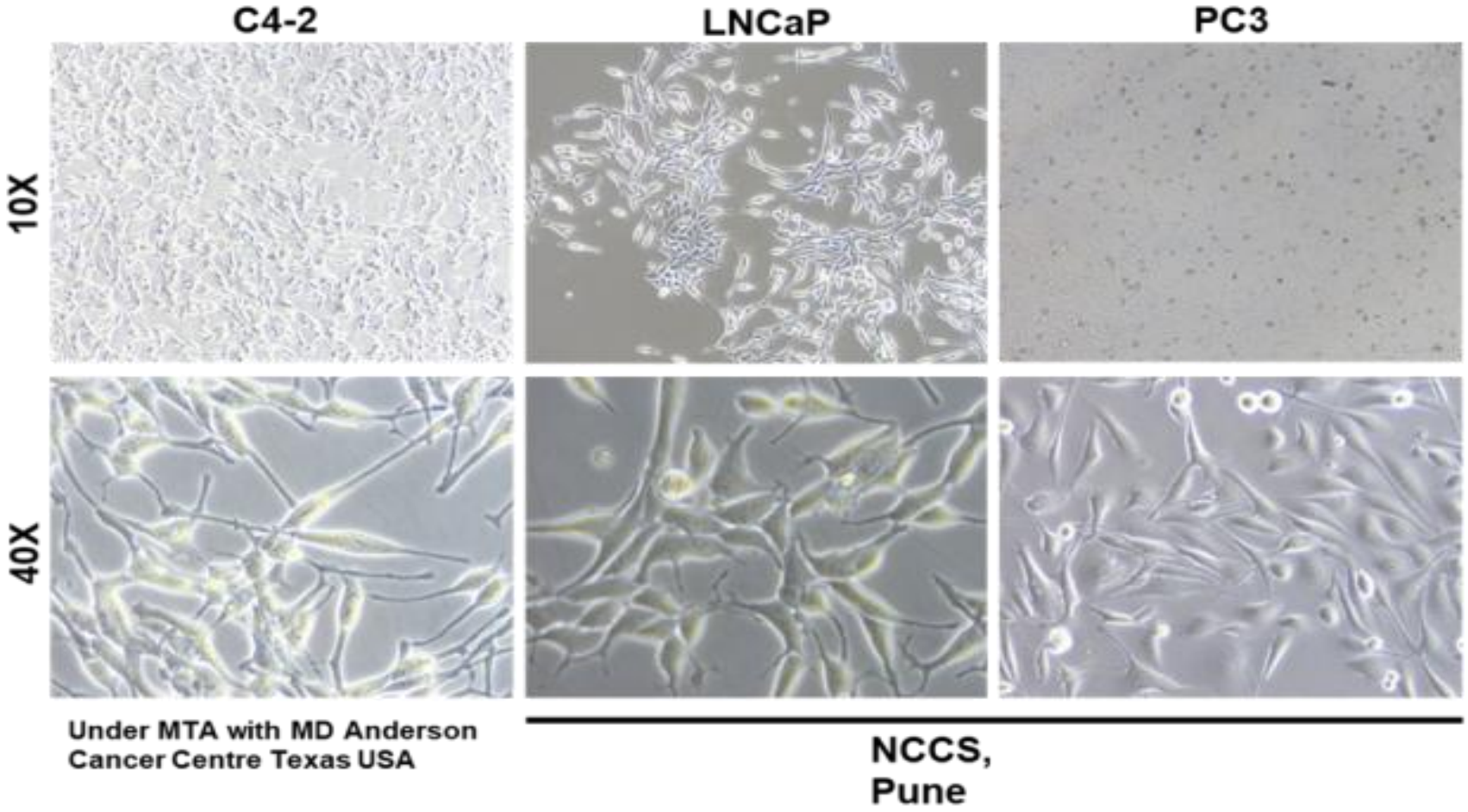
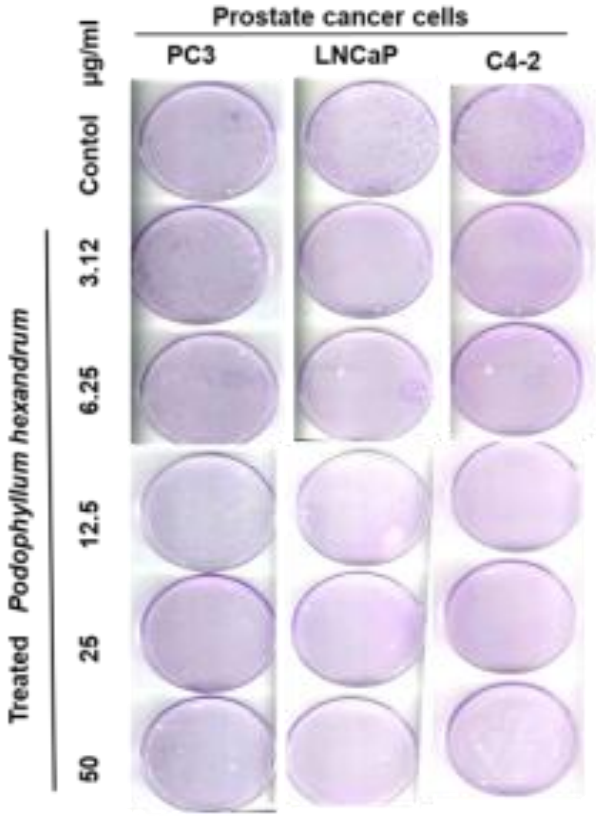


Figure.3 Effect of *Podophyllum hexandrum*on inhibition of CFU in PCa cells.
A.Cells were grown to confluency in RPMI supplemented with 10%FBS,1% L-glutamine,1% Penstrep and treated with different concentrations of leaf extract of *Podophyllum hexandrum*. Colonies were counted 7 days after.
B. Quantification of CFU



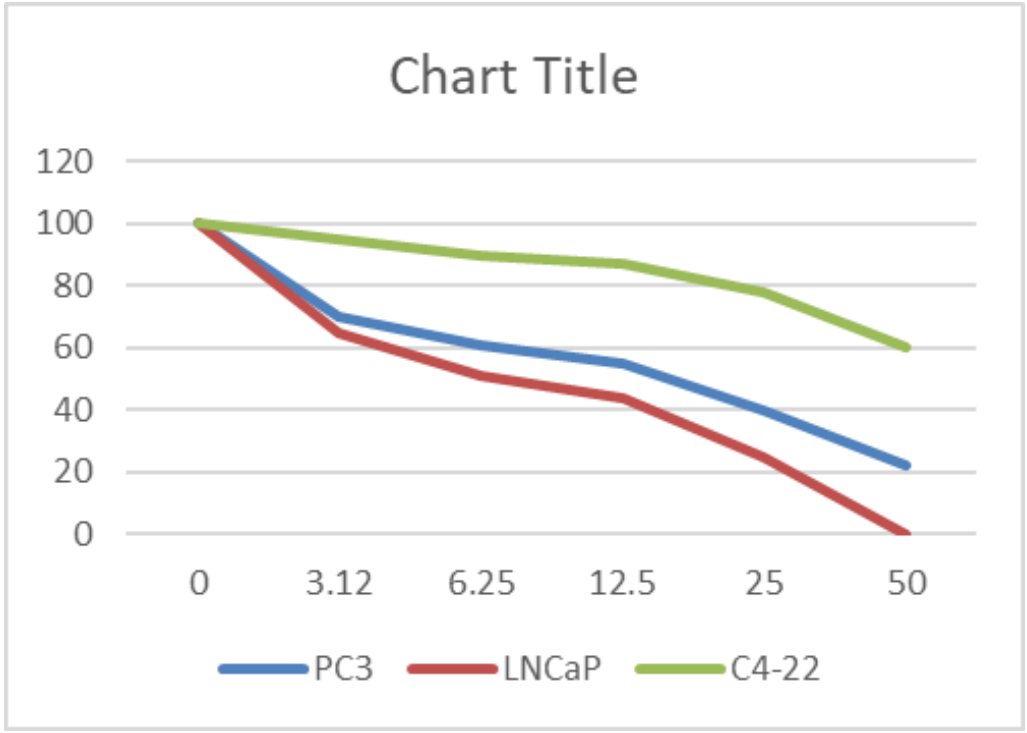
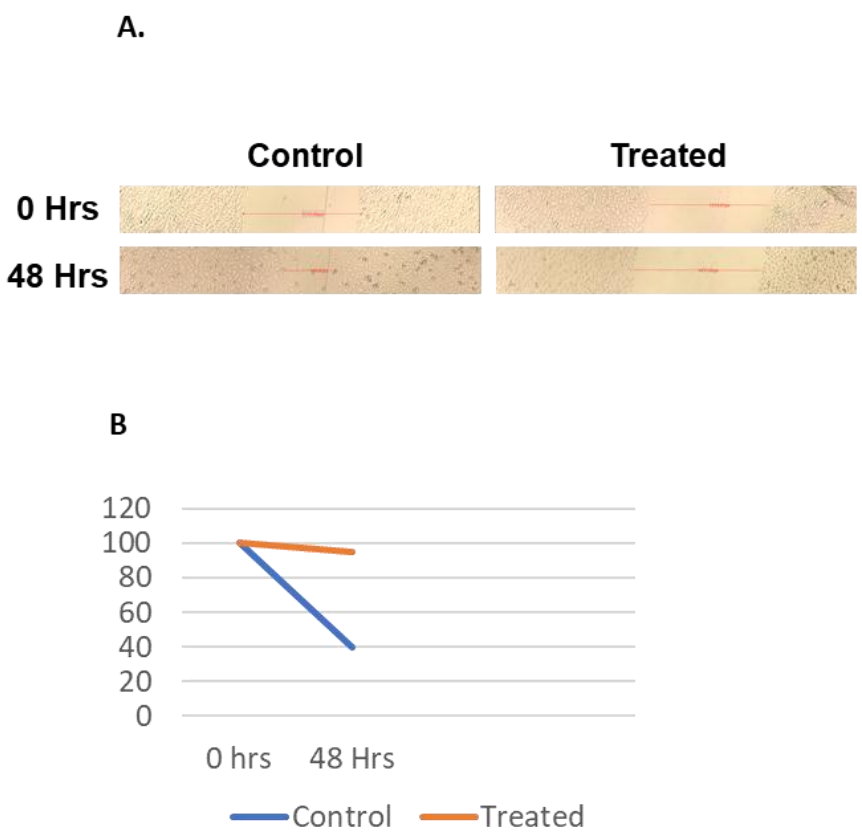


Figure.4 Wound healing assay in C4-2 cells. A. Confluent C4-2 cells were treated with *Podophyllum hexandrum* extract (50µg/ml) after wounding across the cell monolayer with a sterile pipette tip for 48 hrs. Cells were imaged at 0 and 48 hours after treatment and gap widths were measured. There was no significant wound closure after 48 hrs in treated cell lines while there was a near to complete wound closure in untreated cells (control) .B. Quantification of gap closure in A



New approaches for identification of novel small-molecule inhibitors of CRPC proliferation are significant in light of the need to overcome resistance of CRPC cells to first- and second-generation antiandrogens such as flutamide, nilutamide, bicalutamide, and the more recently approved enzalutamide. One of best alternatives to these first- and second-generation antiandrogens could be plant based extracts.

In this study, we screened library of 372 plant extracts for their ability to inhibit cancer cell migration and colony forming units in castration resistant prostate cancer cells through wound healing and colony forming unit assays. We identified leaf extract of *Podophyllum hexandrum* capable of inhibiting CRPC growth and migration.Wound healing assay revealed that in presence of this extract, cancer cells show inhibition of cell movement and no cellular gap closure, thus showing detrimental effect on invasiveness of C4-2 cells. CFU assay depicted inhibition of cellular proliferation by this extract and reduced colony forming units with increasing concentrations in C4-2, LnCaP and PC3 cells. The examinations herein indicate that plant extracts are novel drugs having the ability to inhibit CRPC proliferation. These results are consistent with those of Masoodi et. al. who observed synthetic molecules inhibited the cancer cell migration in CRPC cells (Dar, Masoodi *et al.*, 2014). Thus, *Podophyllum hexandrum* leaf extract can be used for developing natural product drugs as potent therapeutics for CRPC because CRPC patients are on synthesized drugs. Therefore, this investigation provides a wonderful starting point for the development of more effective analogs to combat prostate cancer.

In summary, we have generated a screening protocol for plant-based extracts having the ability of reducing proliferation and cell movement in CRPC cells. Identification of leaf extract of *Podophyllum hexandrum*as antiproliferative and anti-invasive extract in CRPC cells was possible through our investigation. Further characterization studies of this extract may lead to new molecules with the capability to treat CRPC patients. Follow up studies on this aspect could focus on the molecular mechanisms of antiproliferation of cancer cells by plant extracts.

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