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Inhibitory property of chloroform and ethyle extract of *Solanum nigrum* L. whole plant on proliferation of melanoma treated against A375 cell lines

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Abstract
Medicinal plants besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity. This study was intended to evaluate the anti-cancerous activity of chloroform and ethyle acetate extract of *Solanum nigrum* L. whole plant on the human melanoma cancer cell lines (A375).The percentage viability of cancer cell lines was carried out by using trypan blue dye exclusion method and cytotoxicity assay was screened by applying MTT [3-(4, 5-Diamethylthiazol-2-yl)-2,5-Diphenyl tetrazolium Bromide] assay. *Solanum nigrum L.* chloroform extract has depicted momentous and potent cytotoxic effect on A375 cell lines with IC_{50} 346µg/ml in concentration ranging between 50µg/ml to 750µ/ml and acted as best agent to reduce the number of cancer cells to minimum. Further studies are needed to better characterize the mechanism of action underlying the beneficial effects of this alkaloid on their pathologies. Hence *Solanum nigrum L.* can be considered to be important to further investigate on molecular level to ascertain mechanism of action for said activity.

Keywords: *Solanum nigrum* L., anticancerous activity, A375 cell line, MTT assay Trypan blue dye

Introduction
Solanum nigrum L. (Kaambal) (Kashmiri) has been traditionally used to treat pathological ailments like fever, ulcers, bacterial infections, fungal infections, jaundice and liver disorders (Creasy *et al.*, 1981; Capizzi *et al*., 2003; Sudhanshu *et al.*, 2000 and Borgia *et al*., 1981) [2]. The most important constituents from *Solanum nigrum* (i.e., vincristine, irinotecan, camptothecines) and microorganisms (i.e., doxorubicin, dactinomicines, mitomycin and bleomycin) (Grever, 2001) [6]. The history of *Solanum nigrum* L. dates back to ancient China and the Mediterranean region as a highly popular laxative drug and a general tonic (Dashputre *et al.*, 2010) [5]. It is used as purgative and astringent tonic; its stimulating effect combined with apparent properties renders it especially useful in tonic dyspepsia (Chintana *et al*., 2012) [4]. Powdered roots are sprinkled over ulcer for healing. Leaf and berries are eaten either raw or boiled, sprinkled with salt and pepper. Some workers have worked out antitumor activity of *Solanum nigrum* L. (Anindyajati *et al*., 2010) [1] but very little is known about the mechanisms involved. The endeavor of the present study was to travel around the potential anti-cancerous activity of *Solanum nigrum L.* whole plant on melanoma cell lines. The study was not only supportive in determining the optimum dose of solanine toxin employed against melanoma cancer but also in the development of a new and a potential anti-cancer drug. The aqueous chloroform extract of *Solanum nigrum L.* whole plant is an effective and potent anti-cancerous agent against human malenoma cancer cell lines so it may provide a poor man friendly and a drug of preference to the world.

Meterials and methods
Methods of Preparation of Plant Extract
The plant *Solanum nigrum* L. was collected and washed thoroughly under running tap water and then rinsed in distilled water and allowed to dry for some time. Then the plant was shade dried without any contamination for about 3 to 4 weeks. The powder was extracted according to (Rashmi *et al*., 2010).

The dried plant was powdered (coarse) and subjected to Soxhlet appratus using ethyl acetate and chloroform respectively. Almost all the chlorophyll and lipid is deposited on the side of the flask and was removed carefully. The extraction was done with each solvent until the supernatant in the Soxhlet became transparent for 36 hours. Every time before taking the solvents of higher polarity to remove the traces of previous solvents, exhausted marc was completely dried. All the extracts were filtered, dried and weighed.

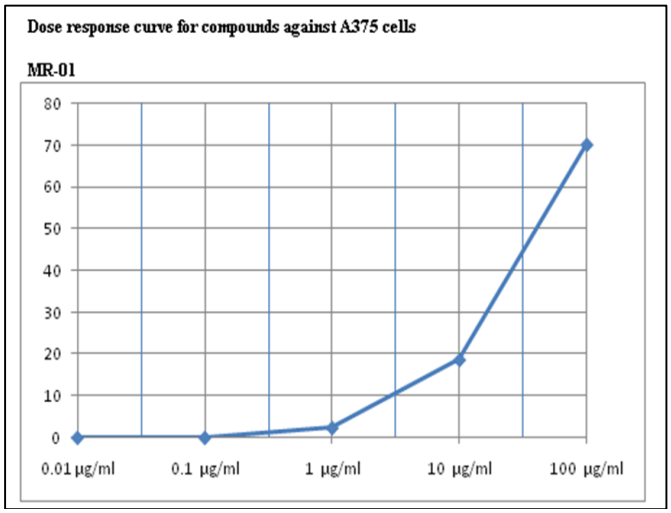
Cell counting was performed by applying trypan blue dye: Calculated the number of cells per ml, and the total number of cells, [15] using the following formula:-
%Viability = (Live Cell Count/ Total Cell Count x100)

Micro culture tetrazolium (MTT) assay Procedure: The monolayer cell culture was trypsinized and the cell count was adjusted to 3- lakh cells/ml using medium containing 10% newborn calf serum. To each well of 96 well microlitre plates, 0.1ml of diluted cell suspension was added. After 24 hours, when the monolayer formed the supernatant was flicked off and 100 µl of different test compounds were added to the cells in microtitre plates and kept for incubation at 37ºC in 5 % CO2 incubator for 72 hour and cells were periodically checked for granularity, shrinkage, swelling. After 72 hour, the sample solution in wells was flicked off and 50µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37oC in 5% CO2 incubator. The supernatant was removed, 50 µl of Propanol was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader
at a wavelength of 490 nm [16]. The percentage growth inhibition was calculated using the formula below: The percentage growth inhibition was calculated using following formula, % CellInhibition=100−{(At−Ab)/(AcAb)}×100
Where, At= Absorbance value of test compound, Ab= Absorbance value of blank, Ac=Absorbance value of control
Percentage cell survival rate was calculated by applying formula, % Cell Servival = {(At−Ab)/(Ac−Ab)}×100, Where,
At= Absorbance value of test compound, Ab= Absorbance value of blank, Ac = Absorbance value of control, % cell inhibition= 100-cell survival.

Result and Discussion

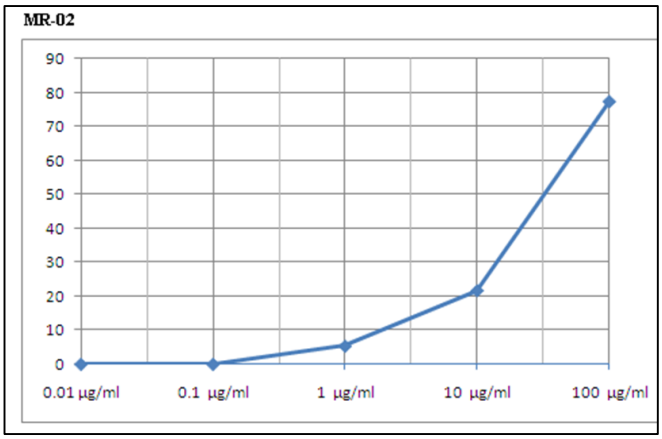
Viability and Characterization of Cell Lines

Cell lines derived from NCCS, Pune were free from any type of bacterial and fungal infectivity. *In-vitro* studies on ethyl acetate and chloroform extract of *Solanun nigrum* L. showed significant antitumor activity against human melanoma cell line (A 375). The viable cell percentage was obtained by performing trypan blue dye exclusion technique and cytotoxicity activity was conceded out by using by MTT assay.
To measure the cytotoxicity of chloroform and ethyl acetate extract of *Solanum nigrum* L. in human cell lines (A375). A375 cells were cultured with chloroform extract (0.01, 0.1, 1, 10, 100 µg/ml) and (0.01, 0.1, 1, 10, 100 µg/ml) with ethyl acetate extract or without extract for 12hrs to 96hrs. IC_{50} value was experimentally calculated to be 55.93 mg/ml for chloroform and 64.67 mg/ml for ethyl acetate extracts. As chloroform extract having low IC_{50} was subjected to further proceeding. Cell viability was evaluated by trypan blue dye exclusion method. By using trypan blue test, chloroform extract exhibited a remarkable reduction against A375 cell viability in a concentration dependent manner, these results are in concordant with MTT assay. Thus inhibition of cell growth by a chloroform extract was more pronounced at concentration of (100ug/ml). On comparing both the extracts, chloroform extract showed high cell viability. *In vitro* screening revealed that the aqueous extract of *Solanum nigrum* L. exhibits significant activity against A375 cell line. The *Solanum nigrum* L. extract showed potent activity against cell viability in a dose dependant manner (Graph 1). Cytotoxic upshot of aqueous extract of *Solanum nigrum* L.against melanoma cell line (A375), when it was being incubated for 72hrs with 3 (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) (Graph 2). IC_{50} value was experimentally calculated to be 55.93 mg/ml for chloroform and 64.67 mg/ml for ethyl acetate extracts. The aqueous extract showed cytotoxicity in a concentration dependant manner. In the present study *in-vitro* screening of both extracts revealed that the percentage cell survival rate decreased significantly at 100µg/ml extract treatment. When the A375 melanoma cells were treated with 1µg/ml cisplatin alone, there was considerable reduce in the percentage of cell survival rate. But when the same cells were treated with 100µg/ml extract adjuvant with 1µg/ml cisplatin, there was remarkable turn down in the percentage of cell survival rate.
Similarly Lin *et al.*, (2005) [10] observed that Solanine is a newly isolated alkaloid which has a strong inhibitory effect on the growth of tumor cells. By using cell cultures, it was demonstrated that the tumor cells were more sensitive to alkaloid than non-transformed cells and that this could be caused by the higher binding affinity of alkaloid to tumor cells than to non-transformed cells. This revealed that both extract were having significant anticancer potential but amongst them chloroform extract was more potent.



Graph 1: Representing significant activity of ethyl acetate extract of *Solanum nigrum* L. against A375 cell line.

The extract showed activity against cell viability in a dose dependant manner. There was considerable decline in cell viability at given extract concentration of 100µg/ml



Graph 2: Representing significant activity of chloroform extract of *Solanum nigrum* L. against A375 cell line.

The extract showed activity against cell viability in a dose dependant manner. There was considerable decline in cell viability at given extract concentration of 100µg/ml.

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