Evaluation of anticancer activity of *Catunaregam spinose and Elytraria crenata* collected from koyananagar wildlife sanctuary

[1]Minal Kulkarni, [2]Dr. Priti Patel

DR.L.H.HIRANANDANI COLLEGE OF PHARMACY

***Abstract*: Ethnopharmacology is a scientific approach to study the substance used medicinally, by different ethnic or cultural groups. Due to urbanization there is decrease in population of traditional herbalist. Hence there is a need to survey, document & conserve all the data related to herbs before it is being lost forever. This newer herbal therapies would lower the adverse and toxic effects of the drug and improves the quality of life.**

***Aim*: The aim of the present study was to identify and document different medicinal herbs used by traditional herbal healers from koyananagar wildlife sanctuary by conducting ethnopharmacological survey in that region & to evaluate anti-cancer activity of *Catunaregam spinose and elytraria crenata* plants.**

***Keywords*: Ethnopharmacology, anti-cancer, crown gall tumor inhibition assay, cell line study.**

INTRODUCTION

Ethnopharmacology is a scientific approach to study the substances used medicinally, especially folk remedies by different ethnic or cultural groups. herbal medicines are being used to alleviate quite a lot of illnesses worldwide, including India, as plants are the basis of life on earth and are central to people's livelihood. Medicinal plants are utilized for the treatment of various diseases and contributed as a foundation of motivation for novel therapeutic agents. Around 80% of the world still depends on the traditional uses of medicinal plants. [1] Though the plants are being used from generations in tribal communities, there is lacuna of systematic documentation of this knowledge. For validation of tribal healer's traditional claims, scientific research on Indian herbs has been intensified day by day. People will be better informed regarding the effective drug treatment and improved health status through the scientific evaluation and proper distribution of the ethnic medical provisions. Scientifically proved pharmacological properties of a lot of herbs give an idea about the presence of a plethora of phytoconstituents which have individual efficacies and also found to possess useful properties in combination with others. Tribal of such areas does not keep any records of healing by the use of natural products but they pass the valuable information verbally from generation to generation. [2] There is decrease in population of traditional herbal healers of tribal areas because of increasing urbanization and the allopathic system of medication gaining more acceptances among current generations. Hence there is need to survey, document and conserve all the data related to herbs being used by traditional herbal healers from generations before it is being lost forever. In order to gather the indigenous data from the study area i.e koyananagar wildlife sanctuary traditional healers were questioned based on several parameters such as the medicinal plants used by them, disease for which the plant is most commonly used, time of collection, scientific name, common name/local name, part used, route of administration, dose and dosage forms, method of preparation, solvent/adjuvant used, soil type, ailments in which they are used, any other use if any, side effects, precautions and contraindications, duration of treatment, frequency of relapse, success rate etc.[3] The data obtained through study was analyzed by systematical classification followed by applying statistical analysis such as use value, choice value, fidelity level, frequency of citations. Results of this classification and statistical evaluation were screened through thorough literature review to find plants with promising use value, choice value etc. but whose pharmacological activity yet not been explored preclinically and further preclinical studies of such plants were performed for their mentioned pharmacology[4].

Cancer is a disease in which abnormal cells divide in an uncontrolled way within the body of aberrational forms of the body's own cells. All types of cancer such as carcinoma, sarcoma, lymphoma, leukemia, melanoma etc. goes through a series of steps described by progressive loss of normal growth control. Cancer can affect people at all ages even fetus; however, the risk for most varieties increases with age. Cancer has an enormous impact on the healthcare economy and represents a great health burden and exhausts healthcare resources worldwide. Most prevalent and common cases of cancers worldwide and in India are – breast cancer, cervical cancer, lung cancer, oral cancer, stomach cancer, and colorectal cancer.[5] Hence, according to prevalence and most common cancers worldwide and in India the selections of cell lines were done. (MCF-7 human breast cancer cell line, HeLa cervical human cancer cell line, A549- human lung cancer cell line).[6]

With the help of data collected through ethnopharmacological survey, and statistical analysis, it was observed that herbalists are using this plant for treatment of tumors, thus shows maximum citation. The dried root of the plants" *catunaregam spinosa" & "elytraria crenata"* has been mentioned in standard reference book of medicinal herbs to possess anti-tumor activity but so far its anti-tumor activity has not been explored pre-clinically. Hence it could be beneficial in the treatment of cancer. Thus the present work was an investigation of the anti-cancer potential of of the plants" *catunaregam spinosa" & "elytraria crenata"* [7]

MATERIALS AND METHODS

Drugs

The Dried fruit Powder of *Catunaregam spinosa(C.S)* and dried root powder of *Elytraria crenata(E.C)*(about 1 tablespoon of powder) 6 gm was boiled with 50 ml of water, boiled for 5 minutes, filtered and the decoction was allowed to cool to room temperature and for every estimation, freshly prepared decoction was used.

Chemicals and reagents

We used Analytical grade chemicals and reagents: Adriamycin, Iodine solution, Chloroform, Sodium hypo chloride, Molisch's Reagent, Benedicts Reagent, Dragendroff Reagent, Fehlings Reagent.

Procurement of bacterial culture

The freeze dried culture of Agrobacterium tumifaciens was procured from MICROBIAL TYPE CULTURE COLLECTION AND GENE BANK [MTCC] Chandigarh-India.

Physicochemical Analysis of Plant:

Total ash value, water soluble and acid insoluble ash value, loss on drying were performed.

Phytochemical analysis [8]

Preliminary phytochemical tests were performed on CS, E.C for detecting alkaloids, glycosides, carbohydrates, proteins, triterpenoids, amino acids, flavonoids, saponins, steroids, and tannins. The confirmatory test for active constituent was also performed.

In-vitro Potato disc assay (Crown gall tumor inhibition assay) [9]

Crown gall tumor inhibition assay used to prescreen the compounds for their antitumor activity because this assay is comparable, rapid, safe, inexpensive and statistically reliable. Crown gall is a neoplastic disease characterized by the transformation of normal plant cells into autonomous tumor cells in a short period of time in many dicots and gymnosperms caused by a gram-negative bacterium, Agrobacterium tumefaciens. A potato disc assay is based on antimitotic activity which can detect a broad range of known and novel compounds with potential antitumor activity against tumors induced by "Agrobacterium tumifacients". A. tumifaciens induced tumors are histologically similar to those found in humans and animals. Because of the tumorigenic mechanisms that are similar in plants and animals, the validity of this assay is predicted.

Evaluation of anticancer activity (SRB Assay) [10]

To evaluate anticancer activity of test compounds using SRB assay at 4 doses level each on 3 cell lines. i.e. (MCF-7 human breast cancer cell line, HeLa cervical Human cancer cell line, A549- human lung cancer cell line). According to prevalence data of cancers in India the selections of cell lines were done.

RESULTS

Physicochemical analysis

Total ash value, water soluble and acid insoluble ash value, loss on drying were performed. Powders are pure as per the permissible adulteration limit.

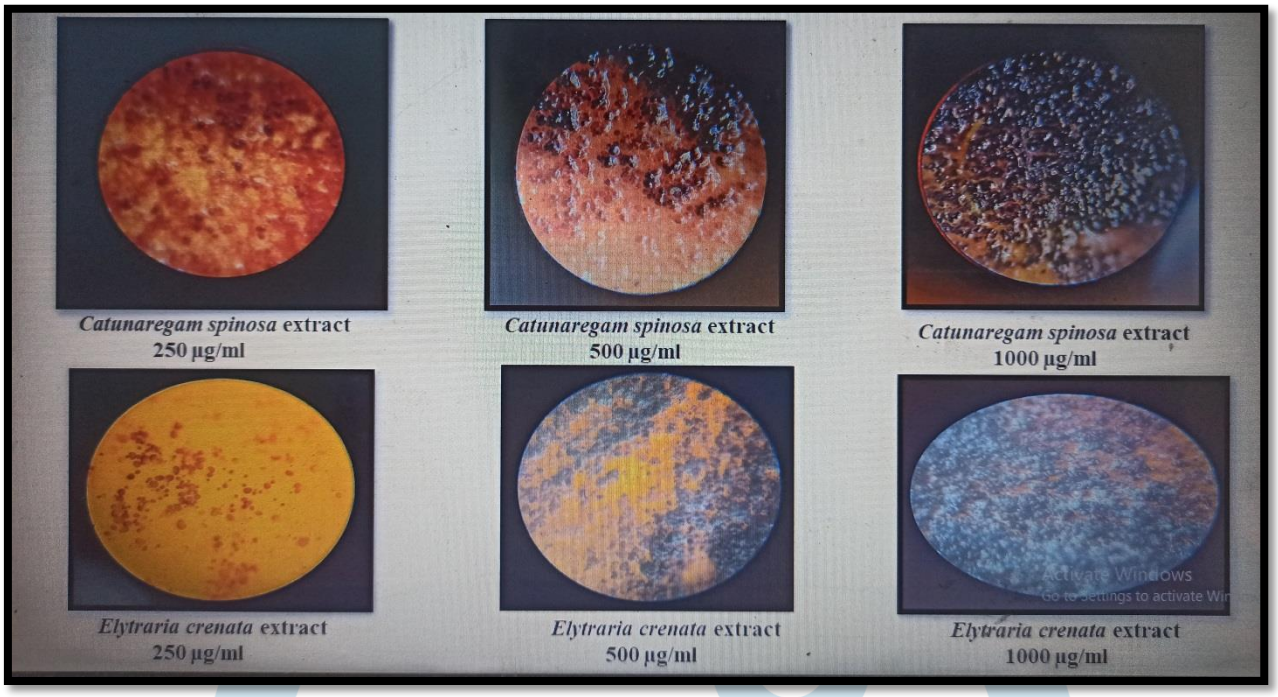
Phytochemical tests

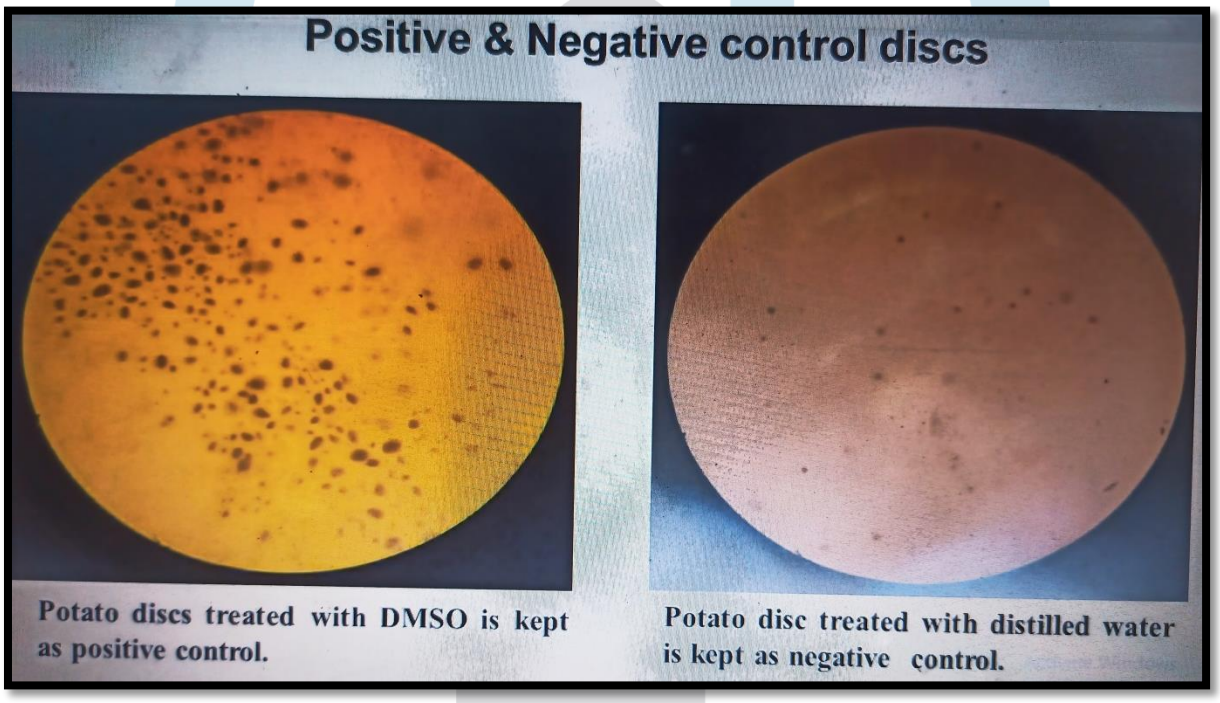
Preliminary phytochemical screening confirmed the presence of Alkaloids, saponins, terpenoids in the C.S & E.C.

In-vitro Potato disc assay Observation

Starch in the potato tissue stains dark blue to dark brown color by the Lugol's reagent whereas tumors produced by A. tumifacients appeared creamy to orange by not taking up the stain.

Result- In anti-tumor activity, the tumor on potato disc was checked by staining the knob with lugol's (I2-KI) solution as agrobacterium induced tumors appear as creamy to orange by not taking up the stain. All concentrations (0.25g/disc, 0.5g/disc and 1.0g/disc) significantly inhibited agrobacterium induced


Catunaregam spinosa extract
250 µg/ml
Catunaregam spinosa extract
500 µg/ml
Catunaregam spinosa extract
1000 µg/ml
Elytraria crenata extract
250 µg/ml
Elytraria crenata extract
500 µg/ml
Elytraria crenata extract
1000 µg/ml


Positive & Negative control discs
Potato discs treated with DMSO is kept as positive control.
Potato disc treated with distilled water is kept as negative control.

Results of cell line studies

1.00	Human Breast Cancer Cell Line MCF-7															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
CS-Sample-1	71.2	60.1	85.3	100.9	61.9	62.9	72.7	103.1	63.5	77.8	59.9	65.7	65.5	67.0	72.6	89.9
ES-Sample-2	53.5	70.5	52.4	51.4	61.8	65.0	56.8	59.9	50.2	63.6	60.6	58.4	55.2	66.4	56.6	56.6
ADR	28.6	22.2	18.9	28.8	28.6	22.2	18.9	28.8	28.6	22.2	18.9	28.8	28.6	22.2	18.9	28.8

MCF-7	LC50	TGI	GI50*
CS-Sample-1	NE	NE	NE
ES-Sample-2	NE	NE	<10

2.00	Human Cervical Cancer Cell Line HeLa															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
CS--1	104.1	112.1	115.8	97.8	102.7	110.9	116.5	99.3	106.8	108.1	115.5	101.7	104.6	110.4	115.9	99.6
ES-2	98.4	96.9	74.0	8.6	100.5	97.7	69.1	8.4	92.9	93.0	66.8	8.2	97.3	95.9	69.9	8.4
ADR	-35.8	9.1	-54.2	-48.8	-35.8	9.1	-54.2	-48.8	-35.8	9.1	-54.2	-48.8	-35.8	9.1	-54.2	-48.8

HeLa	LC50	TGI	GI50*
CS-Sample-1	NE	NE	>80
ES-Sample-2	NE	>80	50.9
ADR	NE	<10	<10

3.00	Human Lung Cancer Cell Line A-549															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
CS -1	72.4	74.7	81.9	144.8	63.3	68.3	75.7	134.4	66.5	70.1	77.0	124.7	67.4	71.0	78.2	134.6
ES-2	55.4	52.8	58.2	66.5	72.3	58.8	56.9	72.0	65.2	52.4	48.5	59.2	64.3	54.7	54.5	65.9
ADR	-32.9	-35.8	-44.2	-32.2	-32.9	-35.8	-44.2	-32.2	-32.9	-35.8	-44.2	-32.2	-32.9	-35.8	-44.2	-32.2

A-549	LC50	TGI	GI50*
CS-Sample-1	NE	NE	NE
ES-Sample-2	NE	>80	NE
ADR	NE	<10	<10

DISCUSSION

With the help of data collected through ethnopharmacological survey, and statistical analysis, it was observed that herbalists are using this plant for treatment of tumors, thus shows maximum citation. The dried root of the plants" *catunaregam spinosa" & "elytraria crenata"* has been mentioned in standard reference book of medicinal herbs to possess anti-tumor activity but so far its anti-tumor activity has not been explored pre-clinically. Hence it could be beneficial in the treatment of cancer. Also it has been mentioned in standard reference book of Indian medicinal plants to possess anti- oxidant activity; hence the aim is to confirm the claim made by herbalist related to anti-tumor potential of this plant. From the literature, it was found to possess antioxidant, anti-septic, anti-inflammatory, hepatoprotective, Antibacterial, Anti-Diabetic. This study aims at exploring efficacy of the dried root of the plants *''catunaregam spinosa" & "elytraria crenata*" as an anti-tumor agent.

In anti-tumor activity, the tumor on potato disc was checked by staining the knob with lugol's (I2-KI) solution as agrobacterium induced tumors appear as creamy to orange by not taking up the stain. All concentrations (0.25g/disc, 0.5g/disc and 1.0g/disc) significantly inhibited agrobacterium induced tumor formation on potato disc when compared to controls with no extract.

To evaluate anticancer activity of test compounds using SRB assay at 4 doses level each on 3 cell lines. i.e. (MCF-7 human breast cancer cell line, HeLa cervical Human cancer cell line, A549- human lung cancer cell line) which shows fair anti-cancer potential. According to prevalence data of cancers in India the selections of cell lines were done.

CONCLUSION

It was observed that the extract of dried fruit powder of *Catunaregam spinosa* and dried root powder of *Elytraria crenata* contains a wide variety of primary metabolites. The indigenous ethno medical data collected from herbalist provided baseline information for further pharmacological investigation of plants *"Catunaregam spinose"* (C.S) and *"Elytraria crenata"* (E.C) demonstrated anti-tumor activity from In-vitro Potato disc assay (Crown gall tumor inhibition assay). It is demonstrated appreciable anti-tumor activity on the cell line assays; it could be taken further to *In vivo* evaluation of anti-tumor activity for confirmation of activity and to explored possible mechanism of action of these plants.

ACKNOWLEDGEMENT

The authors would like to express heartfelt gratitude to Dr. Parag Gide, for facilitating the necessary infrastructure for carrying out the research project.

REFERENCES

[1] Farnsworth, N.R., 1990. The role of ethno pharmacology in drug development. Bioactive compounds from plants, 154, pp.2-21.
[2] . Heinrich, M., Edwards, S., Moerman, D.E. and Leonti, M., 2009. Ethnopharmacological field studies: a critical assessment of their conceptual basis and methods. Journal of Ethnopharmacology, 124(1), pp.1-1
[3] Patil, S., Lavate, R., Shimpale, V. and Rawat, V., Cyclosorus interruptus (Willd.) H. Ito: a new addition to the flora of Maharashtra.
[4] Chanda, S., 2014. Importance of pharmacognostic study of medicinal plants: An overview. Journal of Pharmacognosy and Phytochemistry, 2(5).
[5] Fearon, K., Strasser, F., Anker, S.D., Bosaeus, I., Bruera, E., Fainsinger, R.L., Jatoi, A., Loprinzi, C., MacDonald, N., Mantovani, G. and Davis, M., 2011. Definition and classification of cancer cachexia: an international consensus. The lancet oncology, 12(5), pp.489-495.
[6] . 16. 17. Bézivin, C., Tomasi, S., Lohézic-Le Dévéhat, F. and Boustie, J., 2003. Cytotoxic activity of some lichen extracts on murine and human cancer cell lines. Phytomedicine, 10(6-7), pp.499-503.
[7] Chanda, S., 2014. Importance of pharmacognostic study of medicinal plants: An overview. Journal of Pharmacognosy and Phytochemistry, 2(5)
[8] Yadav, R.N.S. and Agarwal, M., 2011. Phytochemical analysis of some medicinal plants. Journal of phytology.
[9] Nge, P.M., 2016. Antitumor Activity of Aqueous and 70% Ethanolic Extracts by Potato Crown Gall (Pcg) Test or Potato Disc Assay (Pda) Method from Carica Papaya L. Leaves
[10] Orellana, E.A. and Kasinski, A.L., 2016. Sulforhodamine B (SRB) assay in cell culture to investigate cell proliferation. Bio-protocol, 6(21).