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Research Article

INVITRO-ANTICANCER ACTIVITY, ACTIVITY OF ETHANOL EXTRACT OF *GLOCHIDION ELLIPTICUM*

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ABSTRACT

The study was aimed at evaluating the invitro anticancer activity of ethanol extract of leaves of *Glochidion ellipticum* (family: Euphorbiaceae). The various anticancer activity activities were compared to synthetic drugs such as ascorbic acid. When compared with ethanolic extract ethanolic extract showed higher anticancer effect.

INTRODUCTION

In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still likes drugs from plants. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future. Majority of crude herbs come from wild sources and it is collected to assess quality parameters by which presence of various phytochemicals can be confirmed. Standardization of natural products is complex task due to their heterogeneous composition in form of whole plant. Authentication, pharmacognostic evaluation, phytochemical analysis are few basic protocols for standardization of herbals. [1,2]

Since the dawn of the human civilization, the importance of medicinal plants in the treatment of variety of human ailments has been immense. Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. Plants are the essential and integral part in Complementary and Alternative medicine and due to this they develop the ability for the formation of secondary metabolites. Plants are the best source of active secondary metabolites which are beneficial to mankind in treating many diseases (Sandhya. S, 2011).Genus Glochidion have been used for a varied of biological activities in traditional medicine and also have been using by many ethnic groups. It is a vast genus in which many plants explored chemically, but most of the species in this genus were not standardized pharmacognostically. [2,-5]

Detailed literature review states that the plant has broad spectrum of the activities which were claimed traditionally and some are proven scientifically. Most of species in this genus were explored on the basis of the chemical constituent but not on pharmacognostical and pharmacological basis.

Medicinal plants are rich in Secondary metabolites, less in quantity with more value compounds and are potential sources of drugs and essential oils. Many of these compounds are having tremendous values in treatment of various ailments. Traditional herbal practices are now-a-days becoming familiar due to the Natural drugs having no side effects when compared to that of chemical drugs.

Natural products from medicinal plants are known to be chemically balanced, effective and least injurious with none or much reduced side effects as compared to synthetic medicines. Natural products, which come out from medicinal plants are also important for pharmaceutical research and for drug

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development as a sources of therapeutic agents. India is perhaps the largest producer of medicinal herbs and rightly called the botanical garden of the world, which are used for thousands of years in the indigenous system of medicine like Ayurveda, Siddha and Unani. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. Their role is twofold in the development of new drugs either for development of a medicine or used for the treatment of diseases. Since ancient times many plants have been utilized by man for extracting and utilizing secondary metabolite products.

The Euphorbiaceae are mostly monoecious herbs, shrubs, and trees, sometimes succulent and cactus-like, comprising one of the largest families of plants with about 300 genera and 7,500 species that are further characterized by the frequent occurrence of milky sap. The leaves are mostly alternate but may be opposite or whorled and they are simple, or compound, or sometimes highly reduced. Stipules are generally present but may be reduced to hairs, glands or spines. The flowers are unisexual and usually actinomorphic. They may be highly reduced by suppression of parts, in the extreme form consisting of a naked stamen as a male flower and a naked pistil as a female flower. A specialized type of miniature inflorescence called a cyathium occurs in about 1,500 species comprising the genera Euphorbia and Chamaesyce. The cyathium consists of a single naked pistillate flower surrounded by cymes of naked staminate flowers, each consisting of a single stamen. These flowers are all enclosed in a cup-like involucre that typically is provided with peripheral nectaries and petaloid appendages such that the whole aggregation closely resembles a single flower. In other members of the family the flowers and inflorescences are more ordinary in appearance, with male and female flowers typically bearing a 5-merous calyx and corolla of distinct segments, although the corolla is sometimes absent. In these forms the androecium most commonly consists of 5, 10 or sometimes numerous distinct or monadelphous stamens. The gynoecium of female flowers consists of a single compound pistil of typically 3 carpels, an equal number of styles or primary style branches, and a superior ovary with typically 3 locules, each bearing 1 or 2 collateral, axile-apical pendulous ovules. The fruit is usually a capsular schizocarp. [6-7]

MATERIALS AND METHODS

All the chemicals and reagent used were of laboratory grade and were procured from manufactures ofResearch lab fine chemicals, Mumbai., LobaChemie, Mumbai, Sigma-Aldrich, Mumbai., Hi Media Lab Mumbai, Finar reagents, Ahmadabad, Merck, Mumbai, Genuine Chem., Mumbai, Labin, Mumbai, Moly Chem., Mumbai**)**

Collection of plant

The plant was collected from the forest regions of Koyna dam of Karad dist, Satara. It was authenticated by Dr. Sanjay S. Sathe, Asso. Professor, Dept. of Botany, PDVP, mahavidyalaya Tasgaon, Dist-Sangli. A herbarium was prepared and deposited in the Dept. of Pharmacognosy for further reference. The plant was identified as *Gochidionelipticum*. (Euphorbiaceae) and was certified under Voucher No: RCP-SNG/ ph'cog/ 2009-10/003.

Extraction methods

Preparation of various extract of medicinal plants

Aqueous extraction

Aqueous extracts *Gochidionelipticum* were carried out by cold maceration. In this process, solid ingredients were subjected to cold maceration with chloroform: Water I.P (2:98) (**Indian Pharmacopoeia (I.P.); 1996**). Powder was placed in 2 liters round bottom flask for about 7 days at room temperature in a warm place. The flask was securely plugged with absorbent cotton and was shaken periodically with frequent agitation until soluble matter is dissolved. The mixture was filtered and after most of the liquid has drained, the filtrate was concentrated to residue at constant temperature bath at temperature 50^0C.
Note: Chloroform water I.P.

2.5 ml of chloroform was shaken with 900 ml of water until dissolved and diluted to 1000 ml with water.

Successive solvent extraction

The dried leaves of the plant of *Gochidionelipticum* were reduced to coarse powder (40 size mesh) and around 200 gm of powder was subjected to successive hot continuous extraction (soxhlet apparatus) with petroleum ether (60-80^0C), chloroform, ethyl acetate and ethanol to about 10 cycles per batch for 1 batches. The extraction was continued until the solvent in the thimble became clear. Each time before extracting with next solvent the powdered material was dried at room temperature.

After the effective extraction, solvent was distilled off using rotary vacuum evaporator and the extracts were concentrated at low temperatures. The dried concentrated extracts were used for phytochemical investigation, isolation, pharmacological activity. [8,9]

Alcoholic extraction

About 500 gms of fresh air-dried Leaves and stem bark of *Gochidionelipticum* were extracted with ethanol by using soxhlet extractor. The extract was filtered and concentrated with the help of rotary vacuum evaporator.

Determination of Anti-cancer activity by MTT Assay

Human cell line consisting of MCF-7 cell line (Breast cancer cell line), was seeded in 96 well plates and allowed to grow until 85% confluence was reached. Then the medium was removed and extract dissolved in 0.2% DMSO, was mixed with medium in four different concentrations namely 10,100, 500, 1000 µg/ml. for controlled cells medium without drug was added. The cells were seeded duplicates and one plate was assayed after 24 hours of incubation by MTT assay.

RESULT AND DISCUSSION

MTT assay

Human cell line consisting of MCF-7 cell line (Breast cancer cell line), was seeded in 96 well plates and allowed to grow until 85% confluence was reached. Then the medium was removed and extract dissolved in 0.2% DMSO, was mixed with medium in four different concentrations namely 10,100, 500, 1000 µg/ml. for controlled cells medium without drug was

added. The cells were seeded duplicates and one plate was assayed after 24 hours of incubation by MTT assay.

Table no.1 Ethanol extracts of leaves *Glochidionelipticum* against MCF-7 cell line

Sr. no.	Concentration(µg/ml)	O.D.	Percentage of cell cytotoxicity
1	Control	0.476	00
2	Std (5 -FU) 20	0.006	98.73
3	*Glochidionelipticum* 10	0.385	19.11
4	*Glochidionelipticum* 100	0.299	37.18
5	*Glochidionelipticum*500	0.099	79.20
6	*Glochidionelipticum*1000	0.096	79.83

From this Table no.60, it is indicate that antiproliferative activity ethanol extract of *Glochidionelipticum* leaves was shown good significant inhibition as compared to standard drug 5 -FU. The extract concentration 1000 µg/ml showed 79.83 % inhibition than standard 98.73 %.

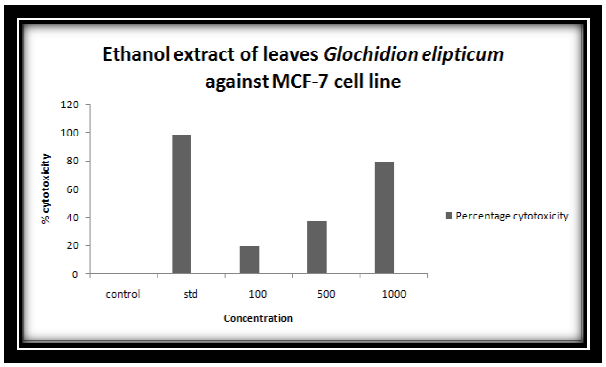

Fig. no. 1 Ethanol extract of leaves *Glochidionelipticum* against MCF-7 cell line

Table no. 2. Ethanol extracts of stem bark *Glochidion elipticum* against MCF-7 cell line

Sr. no.	Concentration(µg/ml)	O.D.	Percentage of cell cytotoxicity
1	Control	0.476	**00**
2	Std (5 -FU) 20	0.006	98.73
3	*Glochidionelipticum* 10	0.210	55.88
4	*Glochidionelipticum* 100	0.123	74.15
5	*Glochidionelipticum*500	0.026	94.53
6	*Glochidionelipticum*1000	0.012	97.47

From this Table no.61, it is indicate that antiproliferative activity ethanol extract of *Glochidion elipticum* stem bark was shown good significant inhibition as compared to standard drug 5 -FU. The extract concentration 1000 µg/ml showed 97.47 % inhibition than standard 98.73 %.

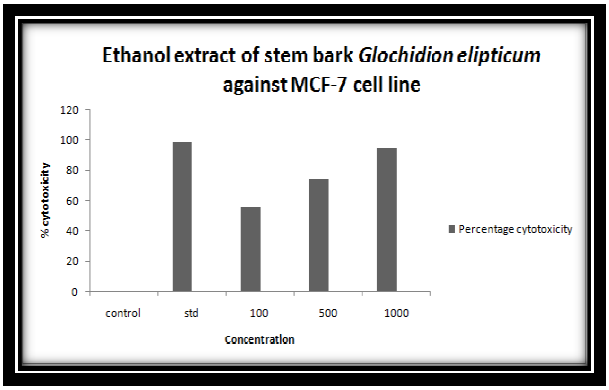

Fig. no. 2 Ethanol extracts of stem bark *Glochidion elipticum* against MCF-7 cell line

Table no. 3 Ethanol extracts of root *Glochidion elipticum* against MCF-7 cell line

Sr. no.	Concentration(µg)	O.D.	Percentage of cell cytotoxicity
1	Control	0.476	**00**
2	Std (5 -FU)	0.006	98.73
3	*Glochidion elipticum* 10	0.205	56.93
4	*Glochidion elipticum* 100	0.115	75.84
5	*Glochidionelipticum*500	0.078	83.61
6	*Glochidionelipticum*1000	0.073	84.66

From this Table no.62, it is indicate that antiproliferative activity ethanol extract of *Glochidionelipticum* root was shown good significant inhibition as compared to standard drug 5 -FU. The extract concentration 1000 µg/ml showed 84.66 % inhibition than standard 98.73 %.

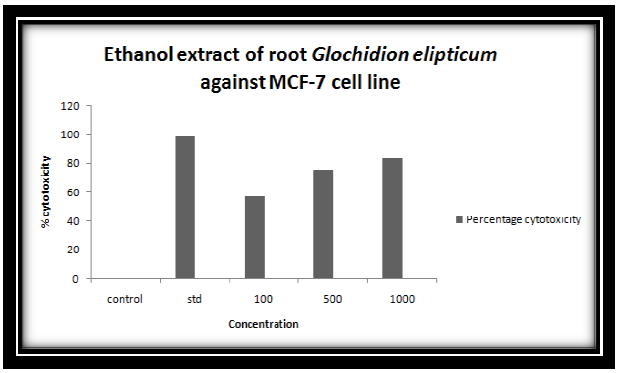

Fig. no. 3 Ethanol extracts of root *Glochidion elipticum* against MCF-7 cell line

Table no.4 Aqueous extracts of leaves *Glochidion elipticum* against MCF-7 cell line

Sr. no.	Concentration(µg)	O.D.	Percentage of cell cytotoxicity
1	Control	0.476	00
2	Std (5 -FU) 20	0.006	98.73
3	*Glochidion elipticum* 10	0.295	38.02
4	*Glochidion elipticum* 100	0.108	77.31
5	*Glochidionelipticum*500	0.079	83.40
6	*Glochidionelipticum*1000	0.046	90.33

From this Table no.63, it is indicate that antiproliferative activity aqueous extract of *Glochidion elipticum* leaves was shown good significant inhibition as compared to standard drug 5 -FU. The extract concentration 1000 µg/ml showed 90.33 % inhibition than standard 98.73 %.

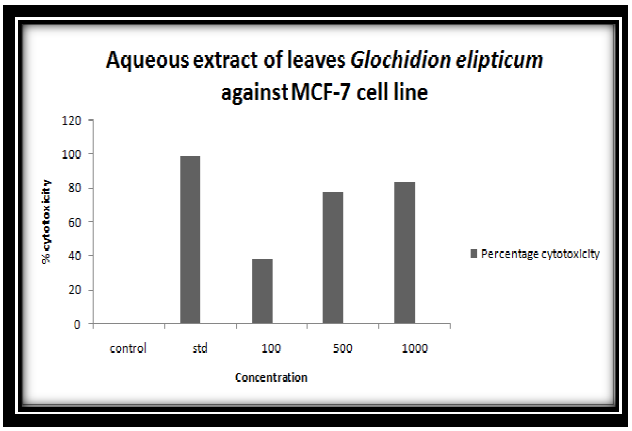

Fig. no. 4 Aqueous extracts of leaves *Glochidion elipticum* against MCF-7 cell line

Table no.5 Aqueous extract of stem bark *Glochidion elipticum* against MCF-7 cell line

Sr. no.	Concentration(µg)	O.D.	Percentage of cell cytotoxicity
1	Control	0.476	**00**
2	Std (5 -FU)	0.006	98.73
3	*Glochidion elipticum* 10	0.159	66.59
4	*Glochidion elipticum* 100	0.135	71.63
5	*Glochidionelipticum*500	0.112	76.47
6	*Glochidionelipticum*1000	0.110	76.89

From this Table no.64, it is indicate that antiproliferative activity aqueous extract of *Glochidion elipticum* stem bark was shown less significant inhibition as compared to standard drug 5 -FU. The extract concentration 1000 µg/ml showed 76.89 % inhibition than standard 98.73 %.

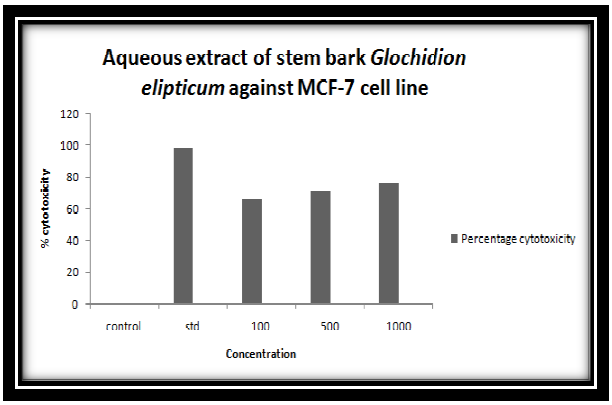

Fig. no. 5 Aqueous extract of stem bark *Glochidion elipticum* against MCF-7 cell line

Table no. 6. Aqueous extract of root *Glochidion elipticum* against MCF-7 cell line

Sr. no.	Concentration(µg)	O.D.	Percentage of cell cytotoxicity
1	Control	0.476	**00**
2	Std (5 -FU)	0.006	98.73
3	*Glochidion elipticum* 10	0.127	73.31
4	*Glochidion elipticum* 100	0.115	75.84
5	*Glochidionelipticum*500	0.105	77.94
6	*Glochidionelipticum*1000	0.082	82.77

From this Table no.65, it is indicate that antiproliferative activity aqueous extract of *Glochidion elipticum* root was shown less significant inhibition as compared to standard drug 5 -FU. The extract concentration 1000 µg/ml showed 82.77 % inhibition than standard 98.73 %.

CONCLUSION

In the present study, the anticancer were evaluated using MMT assay. The various anticancer activity activities were compared to synthetic drugs such as ascorbic acid. When compared with ethanolic extract ethanolic extract showed higher anticancer effect.

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