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Anticancer and Cytotoxic Potential of Ethanolic extract of *Tribulus terrestris* on Hela cell lines

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Abstract

Tribulus terrestris Linn., belongs to family Zygophyllaceae. It is popularly known as puncture vine plant. It is a perennial creeping herb commonly distributed worldwide. The objective of the work is to find out the effectiveness of anticancer potential in ethanolic extract in fruits of T. terrestris. In the phytochemical analysis the ethanolic extract in fruits of T. terrestris showed the presence of Coumarin, Phenol, Phytosterols, Protein, Saponin and Tanins as positive results. The cytotoxic effect was determined by MTT assay and Trypan Blue assay. The cytotoxicity reactivity is moderate in 10µl and 5.0µl and mild in 2.5µl concentration in ethanolic extract in fruits of T. terrestris. The ethanolic extract of T. terrestris given mild to moderate cytotoxicity on L929 mouse fibroblast cells. The results suggested that the existence of bioactive compounds in the ethanolic extract in fruits of T. terrestris revealed a strongest anticancer activity against cervical cancer cell line (HeLa).

Keywords: *Tribulus terrestris,* HeLa cells, MTT assay, Cytotoxicity.

Introduction

Cancer is the common cause of death in human. It is a disease characterized by uncontrolled growth and spread of abnormal cells in humans worldwide[1,2,3]. In general the term cancer is a form of malignant diseases which occurs in many parts of the body. This disease is reflected by a rapid and uncontrolled cell proliferation leading to abnormal growth or tumor which leads to death of the patient[4].

Currently it has been proved that cancer has lot of genetic basis also. There are Oncogenes in humans and under different physical, chemical, environmental, foegraphic, genetic and infective virus factors, the cancer causing genes become active and produce certain type of cancers in human.Cancer detection and diagnosis usually depends on changes in cells and tissues which occur at the nanoscale level inside the cells and are detected either by physical examination or imaging expertise[5].

Cancer occurs in various types. In the several types of cancer, cervical cancer is a Carcinoma of the cervix. It is the 2nd common cancer in women worldwide. The International Agency for Research on Cancer promotes several awareness programmes related to cervical cancer prevention, diagnosis. Nowadays the Pap test is best diagnosis and cost effective practice of cervical cancer[6]. The highest number of women was affected by cervical cancer in India. It was estimated about 1,32,000 cases and 74,000 deaths occurred[7]. The world status of Cervical cancer stands the second major cause of cancer death in the women[8,9]. Human papilloma virus (HPV) occurs in sexually transmitted infection is the important fact of cause of cervical cancer. There are nearly 100 HPV types, 18 have been categorized as high-risk types for cervical cancer and the rest are low-risk types[6]. HPV 16, 18, 31 and 33 have been attributed to be the major risk factors for cervical cancer, HPV-16 and 18 account for almost 70% of the cancers[10].

In India the high risk of cervical cancer occurs due to early age, multiple sexual partners, multiple pregnancies, poor genital hygiene, smoking use of oral contraceptives, religion, ethnicity, etc.[11]. To develop the consciousness of the cancer in public to be based on the treatment, prevention of the cytotoxic activity. There is a need of naturally available new compounds that with inhibit the cytotoxic activity as the treatment of cancer.So the need of alternative source as anticancer drug by naturally occurring source as a remedy usage of plant extracts. Phytochemical examination has been making rapid progress and herbal products are becoming popular as sources of plausible anticancer compounds[12].

Tribulus terrestris belong to family *Zygophyllaceae*. It is a shrub, commonly named as Gokhru, puncture vine and goathead, etc., *T.terrestris* was used as rejuvenation tonic, headache, eye problems, therapy for various diseases affecting kidney, liver, cardiovascular, immune systems and also maintenance of Blood pressure, rib pain[13,14]. It was also used as effective immunomodulating activity and aphrodisiac properties[15,16,17]. Phytochemical compounds present in *T.terrestris* are saponins, alkaloids and flavonoids etc.,[18]. Saponins present in *T.terrestris* can be used as inhibitory effect on breast cancer cell line[19].

The present work was aimed to focus the effect of the ethanolic extract in fruits of *T.terrestris* to combat the dreadful disease –

Carcinoma of cervix using Human cervical cancer cell line (HeLa) (*in vitro* study).

Materials and Methods

Collection of sample: Fruits of *Tribulus terrestris* was collected from Arachalur Erode, Tamil Nadu, India and the specimen was identified and authenticated by Botonical Survey of India, Coimbatore, (BSI/SRC/5/23/2015/Tech/669 and 1824). The specimen was stored in Department of Biochemistry, Bharathidasan College of Arts and Science, Erode, Tamil Nadu, India.

Preparation of samples extract: The fruits of *T.terrestris* sample was collected, shadow dried and fine powder. Using Soxhlet's apparatus the *T.terrestris* sample powder was extracted with various solvents (petroleum ether, chloroform, ethanol and aqueous) based on increasing polarity for 72 hours. Ethanol is high polarity solvent when compared with other solvents, so ethanol solvent was used for the extraction of secondary metabolites from the fruits of *T. Terrestris*. Thus the ethanolic extract in fruits of *T. Terrestris* was used for phytochemical assay and anticancer activity.

Phytochemical assay: The phytochemical analysis of various extracts in fruits of *T.terrestris* was tested for the presence of various phytochemicals such as acids, alkaloids, anthocyanins, anthroquinones, couumarin, flavonoids, glycosides, phenol, tannins, protein, reducing sugar, saponins, terpenoids, sterols, triterpenes, saponins alkaloids, carbohydrates, flavonoids, amino acids and fats as per the method of Phytochemical Methods[20].

Anticancer Activity: Celline Culture: HeLa Cervical Cancer cell line was purchased from National Centre for Cell Sciences Pune. HeLa Cells was cultured and maintained in essential medium (Foetal bovine serum).

Cytotoxicity by MTT Assay: Cell growth inhibition was determined by 3-(4,5- dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) assay[21]. *Invitro* cytotoxicity test was carried out for the ethanolic extract in fruits of *T.terrestris* as per ISO 10993:5. The culture medium from the HeLa monolayer was replaced with fresh medium. The ethanolic extract in fruits of *T.terrestris* with different concentration (10µl, 5µl, and 2.5µl) in triplicates were added on the cells and incubated in CO_2 at 37±1 ^oC. At the end of 18 h of incubation, MTT (5 mg/mL) were added in all the wells and continued incubation for 4 hrs. After incubation, DMSO were added in the wells as control and read at 570 nm using photometer.

Cytotoxicity and cell viability were calculated by below formula.

Cytotoxicity = Control – Treated/ Control x 100

Cell viability= Treated / Control x 100

Trypan Blue Assay: The The HeLa cells were grown in Minimum Essential Medium (MEM) supplemented with 10% Fetal bovine serum (FBS). Equal volume of Trypsin (0.25 %) / Ethylenediaminetetraacetic acid (EDTA) (0.02 %) and versene (0.1 %) was used to detach the cells and observed them under a microscope to confirm complete dissociation of the cells. The cells were centrifuged to remove EDTA and resuspended in MEM. 1 x 105 cells were seeded in the well and incubated at 37°C for 24 hrs. Samples were added in different concentration (10µl, 5µl, 2.5µl) in duplicate, Cells grown in the well without sample served as control and the plate was kept for further incubation of 24 hrs. After incubation medium was completely removed and rinsed with PBS. 20 µl of 0.4% Trypan blue were added in each wells and observed under inverted phase contrast microscope (40 X)[22].

Results and Discussion

Phytochemical assay in fruits of *Tribulus terrestris:* The phytochemical screening of solvents extracts of extracts in fruits of *T. terrestris* showed the presence of various phytochemical compounds. In petroleum ether extract, sterols, saponins, alkaloids, resins and glycosides were tested positive. Sterols, triterpenes, alkaloids, glycosides, fixed oils and fats, coumarin glycosides and cardiac glycosides were tested positive in chloroform extracts. In ethanol extract sterols, triterpenes, saponins, carbohydrates, flavonoids, lactones, amino acid and protein, coumarin glycoside showed positive results and others showed negative results. In aqueous extract triterpenes, flavonoids, glycosides, gums and mucilage, coumarine glycosides, cyanopheric glycosides and cardiac glycosides showed positive results and others showed negative results (Table-1 and Figure-1).

In this study the ethanolic fruits extracts *T.terrestris* showed the presence of Alkaloids, coumarins, glycosides, phenol, phytosterols, protein, saponins and tannins. *Boesenbergia rotunda* showed the presence of Quercetin which is a major flavonoid that can reduce apoptosis which showed anticancer activity[23]. Polyphenolic compounds also showed cytotoxic effect against cancer cells by inhibiting mitosis at telophase stage[24].

Cytotoxicity by MTT assay: MTT assay is based on the reduction of MTT (3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide) by mitochondrial dehydrogenase to purple formazan product. Ethanolic extract in fruits of *T.terrestris* were found to be cytotoxic towards Hela cells. The anticancer activity was found significant in the MTT assay shown in the Table-2.

The Ethanolic extract of *T.terrestris* showed 74% in 10µl, 72.7% in 5µl and lesser of 63.4% in 2.5µl concentration of sample. The cell viability was found to be 26% for 10µl, 27.3% for 5µl and 37 ethanolic extract of *T.terrestris*. The cytotoxicity reactivity is moderate in 10µl and 5µl and mild in 2.5µl of ethanolic extract of *T.terrestris*. The ethanolic extract in fruits of

T.terrestris given mild to moderate cytotoxicity on L929 mouse fibroblast cells. The ethanolic extract in fruits of *T.terrestris* has showed the presence of antioxidant activity, wound healing property and cytotoxic activity. The present works suggest the presence of bioactive compounds present in the ethanolic extract in fruits of *T.terrestris* has showed decrease cytotoxic activity increase in antiproliferative against HeLa cervical cancer cell line.

Table-1
Phytochemical analysis of ethanolic extract in fruits of *T.terrestris*

Phytochemical compounds	*T. terrestris*
Acids	-
Alkaloids	+
Anthocyanins	-
Anthroquinones	-
Coumarin	+
Flavonoids	-
Glycosides	+
Phenol	+
Phlobatannins	-
Phytosterols	+
Protein	+
Reducing sugar	-
Saponins	+
Tannins	+
Terpinoids	-

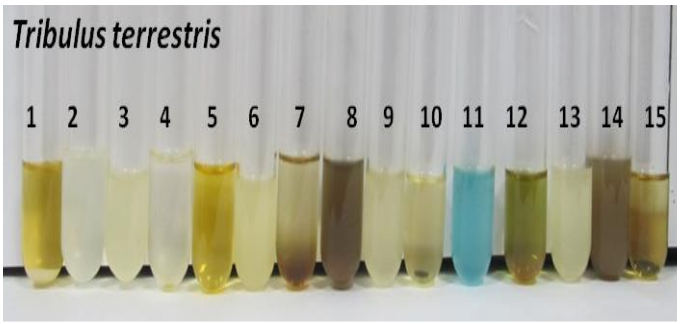

Figure-1
Phytochemical analysis of ethanolic extract in fruits of *T.terrestris*

Table-2
Cytotoxicity and Cell viability of ethanolic extract in fruits of *T.terrestris*

Concentration µl	Cytotoxicity (%)	Cell viability (%)	Cytotoxic reactivity
Control	4.6	95.4	Nil
10	74	26	High
5.0	72.7	27.3	High
2.5	63.4	37.6	Moderate

In the aqueous extract of *T.terrestris* affect survival of normal and cancerous cell lines at a dose dependent manner and IC50 was different between normal and abnormal cells. The anticancer effects of *T.terrestris* on mice sarcoma categories (ASC), breast cancer (Bcao- 37), liver cancer (BEL-7402) and SK-mel, KB, BT- 549, SK-OV-3 tumor cell lines have been reported and these studies confirm our results [25-28].

Similar results were studied in the methanol extract of TT IC50 value was 160 mg/mL. The water extract also showed estrogenic activity at concentrations 27 mg/mL, while the other T. terrestris extracts had anti-estrogenic properties[29]. In another study it was reported that the saponins, structurally similar to diosgenin, present in *T.terrestris* extracts, might block the cell cycle, suppress proliferation, and induce apoptosis in human sarcoma cell lines[30].

Tryphan Blue assay: The Cell culture was maintained by NCCS Strain (HeLa) celline. HeLa is an established and well characterized mammalian cancer cell line that has demonstrated reproducible results and Culture medium with Minimum essential medium supplemented using foetal bovine serum. The Trypan Blue staining was carried out with varying Ethanolic Extraction of *T.terrestris* (Figure-3 and Figure-4).

As per the procedure, the ethanolic extract of TT (5µl and 2.5µl) showed less dead cells indicate none cytotoxic reactivity to HeLa cells after 24hrs contact. The TT (10µl) showed more dead cells that indicates and severe as cytotoxic reactivity to HeLa cells. Control gave none cytotoxic reactivity as expected.

In the previous study the anticancer properties of *T.terrestris* have been reported on various celllines i.e. mouse sarcoma 180 (ASC), Bcap-37 breast cancer cell line, BEL- 7402 liver cancer cell line, SK- MEL, KB, BThavefound that various triterpene alcohols and 549 and SK- OV-3[25,31-33]. *T.terrestris* is a rich source of saponins that its anti-proliferative effect has been proved on Hela-60 cell line. By thisfact, anti-proliferative effect of *T.terrestris*, showed in this study, may depend on saponin constituents[34].

Our results indicated that *T.terrestris* extract has proliferative effect on cervical cancer cell lines significantly. One of the major anticancer treatments, that used alone or in combination with other therapy, is chemotherapy. Major problem of chemotherapy is bone marrow toxicity and immune system suppression. Drugs with proliferative effect on hematopoietic progenitors could reduce these side effects. One strategy to reduce cancer-therapy toxic effects on normal cells, are stimulating drugs such as cytokines or stimulating factors. These agents could stimulate hematopoiesis, but have adverse effects like fever, atopic cutaneous reactions, eczema, diarrhea, bone pain and psoriasis[35].

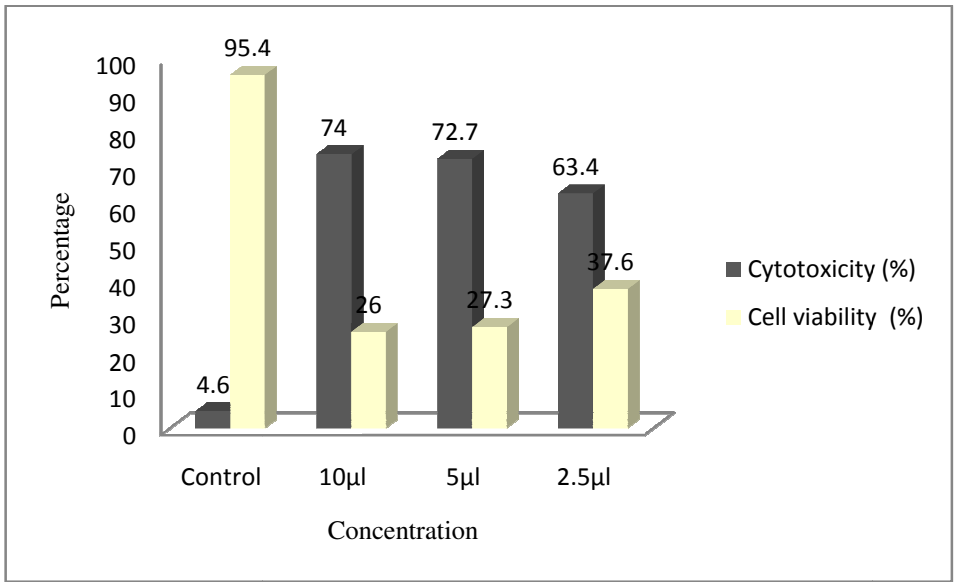

Figure-2
***Invitro* cytotoxicity for *T.terrestris* by MTT Assay**

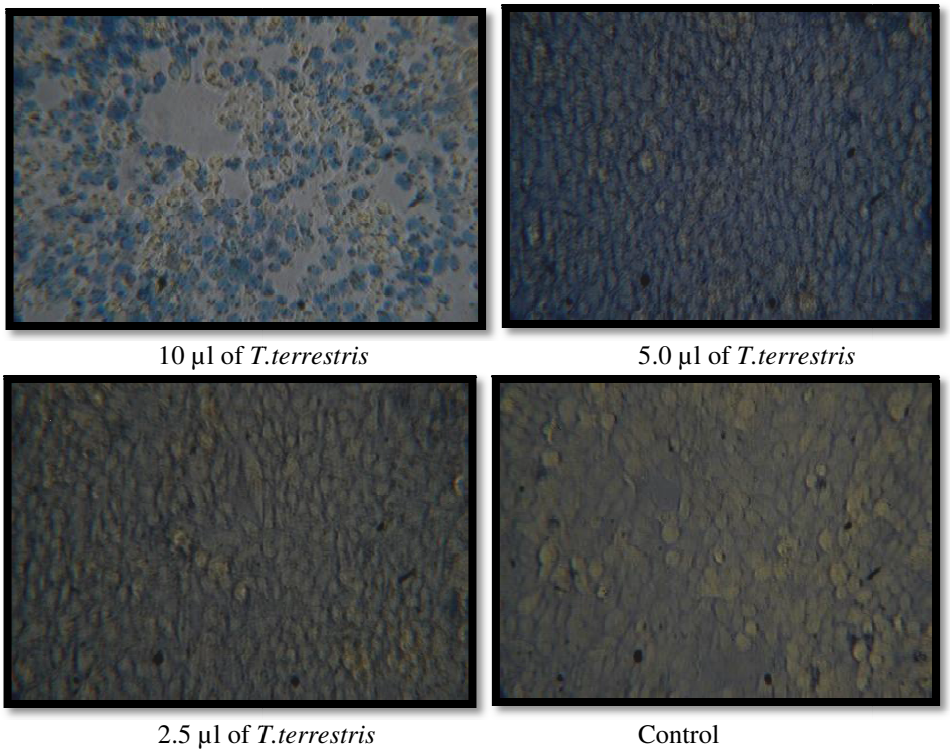

Figure-3
Dead cells represent Nucleus stained with trypan blue and viable cells represent in Nucleus without stain

Conclusion

T.terrestris plant has been traditionally used for large number of pharmacological actions with varying medicinal properties. The recent scientific research related to *T.terrestris* plant showed an enormous biological, medicinal, therapeutical potential of different system of medicine. Front the current research it was concluded that the *T.terrestris* fruits extract found to be better profile as a natural source of systematic treatment of anticancer activity due to the presence of bioactive constituents.

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