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Analysis of anti-cancer potential of *Terminalia arjuna*

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Abstract

The present study was designed to evaluate the anti-cancer potential of petroleum-ether bark extract of *Terminalia arjuna* against cancer cell lines. An *in vitro* screening was performed against two human cancer cell lines namely HEP2 (liver) & HT29 (colon) using Sulforhodamine B (SRB) assay. The activity was tested using 100 µg/ml of the crude plant extract. It showed 78% & 79.33% of growth inhibition against HEP2 & HT29 cancer cell lines, respectively. The plant extract showed significant results against HT-29 and HEP-2 cancer cell lines. The experimental evidence obtained in our study suggested presence of active anti-cancer ingredients in *T. arjuna* that need to be purified and characterized for its possible use in anti-cancer therapy.

KEY WORDS: *Petroleum ether, Cell lines, Sulforhodamine B, Ingredients, Anti-cancer.*

1. Introduction

Cancer is defined as an uncontrolled cell growth and spread of abnormal cells, associated with deregulation of apoptosis. Currently, one death out of four in the United States is due to cancer. When ranked within age groups, cancer is one of the five leading causes of death amongst both males and females and the single largest cause of death worldwide. By 2015 cancer morbidity may climb to around nine million world-wide [6]. Cancer is one of the most severe health problems. The most common types of cancers are lung, stomach, colorectal, liver, breast, prostate etc. Among these the lung cancer is the most common cancer diagnosed in men and breast cancer is the most common cancer diagnosed in women. An estimated 12.7 million people were diagnosed with cancer across the world in 2008, and 7.6 million people died from the cancer during the same year [1]. Current research shows that plants contain a diverse group of more valuable and readily available resource of bioactive metabolites, e.g. alkaloids, tannins, steroids, essential oils and flavonoids [6,12], which have been used in medicinal practices for a long time [16]. Ayurveda, a traditional system of Indian system of medicine mainly based on plant drugs has been successful since very early times for preventing or suppressing the diseases [7]. Plants are being used as indigenous cure in traditional system of medicine for treatment of various types of diseases including cancer [10]. Epidemiological studies suggest that consumption of diets containing fruits and vegetables which are the major sources of phytochemicals, macro and micronutrients reduce the risk of developing cancer [2]. Recently, a greater emphasis has been given towards research on complementary and alternative medicine that deals with cancer management[14]. *Terminalia arjuna* (Family-Combretaceae) is commonly known as Arjun in India. It is a large tree, cylindrical trunk, smooth grey bark and about 10 – 30 m in height. Its leaves are usually sub-opposite, oblong, dark green and 10 -20 cm long. The flowers are yellowish-white while the fruits are 2.5 - 5.0 cm

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ovoid or ovoid-oblong, fibrous. It is commonly grows on the rivers bank, streams and dry watercourses in sub- Himalayan area, West Bengal as well as in central and south India. The bark contain a crystalline compound, arjunine, arjunetin, lactone, reducing/non reducing sugar, essential oil etc. Besides these, it also contains calcium carbonate (34 %), other salts of calcium (9%), tannin, aluminum, magnesium, organic acids (13%), colouring matter and other substances [4].

The present study was therefore planned to evaluate the presence of bioactive compounds and anticancer potential of *T. arjuna bark* extracts against some common human cancer cell lines. Although, many benefits of *T. arjuna* have been claimed but only few of them are scientifically authenticated.

2. Materials and methods

2.1 Plant material and Preparation of plant extract

The plant bark were collected in the months of March to June -2012 from Botanical survey of India and Forest Research Institute Dehradun, Uttarakhand, India . Bark was collected from the trunk of trees and was dried at room temperature in the laboratory and then grind, powdered. The extract was extracted by glass Soxhlet apparatus using petroleum-ether as a solvent. Extract was filtered, dried and stored at low temperature. Extract was re-dissolved in Dimethylsulphoxide (DMSO) to form stock solutions, which were filter sterilized (0.2µm) before testing on cancer cell lines.

2.2 Protocol for Preliminary Screening of Bioactive Compounds

Biochemical tests for the screening and identification of active components have been done using standard protocols. For each test, 100 µg/ml concentration of each extract were used for analysis.

Test for protein

1 ml extract solution were treated with few drops of concentrated nitric acid, formation of yellow color indicated the presence of proteins.

Test for Alkaloids

Extract were dissolved individually in diluted Hydrochloric Acid (HCl) and filter, then filtrate were treated with saturated picric acids resulting the formation of brown precipitate indicated the presence of alkaloids.

Test for Steroids

1 ml Extract was mixed with 2 ml of chloroform then formation of reddish brown color after carefully addition of H_2SO_4, indicated presence of steroids.

Test for Flavonoids

(a) NaOH Test: 100 µl Extract solution was treated with few drops of sodium hydroxide solution, then formation of yellow color was observed which became colorless on addition of dilute HCl acid, indicating the presence of flavonoids.

(b) Lead Acetate Test: To 1 ml extract solution, few drops of lead acetate solution was added.

Formation of yellow precipitate showed the presence of flavonoids.

Test for Phenols: 100 µl Extract solution mixed with 2 ml of 2% solution of $FeCl_3$. Blue/green color resulted indicating the presence of phenols.

Test for Tannins: 100 µl Extract solution mixed with 2 ml of 2% solution of $FeCl_3$. Black color indicated the presence of tannins.

Test for Terpenoids

100 µl Extract solution mixed with 2 ml of chloroform. Then 2 ml of concentrated Sulfuric acid was added carefully and shaken gently. Reddish brown color indicated the presence of terpenoids.

2.3 Cell lines

The liver (HEP 2) & colon (HT 29) cancer cell lines were grown in RPMI-1640 growth media with 2 mM L-glutamine at the pH 7.2. The Antibiotics Penicillin was dissolved in PBS and sterilized by filtering through filter paper (0.2µ) in laminar air flow hood. The whole medium was kept in refrigerator and stored at low temperature (2-8^{0}C). Complete growth medium contained 10% FCS. The medium for cryopreservation contained 20% FCS and 10% DMSO in growth medium. The cell lines were maintained at 37C^0 in a 5% CO_2 atmosphere with 95% humidity [13].

2.4 In vitro study of anticancer activity

The anticancer activity was determined by evaluating the cytotoxic potential of the pre-prepared test material using human cancer cell lines that were allowed to grown on 96 wells tissue culture plates in the presence of test material. The cell growth was measured using ELISA reader after staining with Sulforhodamine B dye (SRB) which binds only to the basic amino acid residues in the trichloroacetic acid (TCA) fixed cells. Mitomycin-C was used as positive control.

2.5 Preparation of Cell suspension for assay

Cancer cell lines were grown in multiple tri-conical flasks (TCFs) at 37^{0}C in an atmosphere of 5% CO_2

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and 90% relative humidity in complete growth medium to obtain enough number of cells. The flasks with cells at sub-confluent stage were selected. Cells were harvested by treatment with Trypsin-EDTA. Cells were separated to single cell suspension by gentle pipetting and the viable cells were counted in a hemocytometer using trypan blue. Cell viability at this stage should be >97% and viable cell density was adjusted to 5,000 - 40,000 cells/100µl depending upon the cell line**.** Cell suspension (100µl) together with 100µl of complete growth medium was added into each well. The plates were kept inside CO_2 incubator at 37^0C for 24 hours in 5% CO_2 atmosphere and 90% relative humidity. After 24 hours, the test material, DMSO (vehicle control) and positive control were added **[9].**

2.6 Sulforhodamine B (SRB) assay

The anti-proliferative SRB assay was performed to assess growth inhibition. This is a spectrophotometric assay which estimates cell number indirectly by staining total cellular protein with the Sulforhodamine B (SRB)**[12]**. The microtiter plates were taken out after 48 hours incubation of the cells with test materials and gently layered with chilled 50% TCA in all the wells to produce a final concentration of 10%. The 96 wells tissue culture plates were incubated at 4°C for 50-60 minutes to fix the cells attached to the bottom of the wells. The supernatant was then discarded. The plates were washed 5-6 times with double distilled water to remove TCA, growth medium, serum proteins, low molecular weight metabolites etc. Plates were air dried and stored until further use. SRB solution was added to each well of the plates and incubated at room temperature for about 30 minutes. The unbound SRB was removed quickly by washing the wells 5-6 times with 1% dilute acetic acid and then air dried. 100µl of Tris buffer (0.01 M, pH 10.4) was added and shaken gently for 5 minutes on a mechanical shaker. Optical density was recorded on ELISA reader at 540 nm.

2.7 Calculations

The Cell viability and growth in the presence of test material was calculated as:

Percent growth in presence of test material:

= (Growth in presence of test material/ Growth in absence of test material) X 100

Percent growth inhibition in presence of test material was calculated as:

= 100 – % growth in presence of test material

2.8 Criteria for Determination of Activity

The test sample/s showing growth inhibition of >70% at 100 µg/ml were considered to be active.

The experiments were carried out and results were expressed as mean ± S.D value. Total data of the experiments were summarized and analyzed by Microsoft Excel 2007.

3. Results and discussion

3.1 Presence of bioactive compounds

Presence of various bioactive compounds in crude extract of *T. arjuna* bark extracts was evident from the current study. Samples showed positive results for the presence of phenolics, alkaloids, flavonoids, terpenoids and tannins **(Table No 1).**

3.2 *In vitro* anticancer activity

Cancer cell lines were grown in RPMI-1640 medium and SRB assay was performed for the test. *In vitro* anti-cancer activity of plant extract sample was performed against liver cancer cell line (HEP 2) showed 76%, 78%, 82%, 78% &76% growth of inhibition of the sample UTU 3, UTU 5, UTU 11, UTU 12 & UTU 17 respectively while Mytomycin-C showed 82% growth of inhibition, where as in case of colon cancer cell line (HT 29), showed 84%, 80%, & 74% growth of inhibition of the test sample UTU 3, UTU 12, & UTU 17 respectively, while Mytomycin-C showed 78% growth of inhibition. The statistical analysis (mean ± S.D.) showed growth of inhibition against HEP 2 and HT 29. The Student's *t*-test showed significant result at $P < 0.05$.

3.3 Discussion

Medicinal plants were main source of therapeutic agents from ancient time to cure many diseases. The observations from the table -1 showed that the *T. arjuna* L. have rich source of bioactive organic compounds like proteins, phenolics, alkaloids, flavonoids, terpenoids, tannins etc. that possess unique medicinal activities. The anti-cancer potential was determined by the cytotoxic potential of the test material using liver and colon cancer cell lines (HEP-2 & HT-29) which are allowed to grow on tissue culture plates in the presence of test material. The cell growth of the medium was measured on ELISA reader after staining with SRB dye which binds to basic amino acid residues in Trichloro-acetic acid fixed cells.

In the present investigation, *T. arjuna bark* extract markedly reduced the cell viability in a

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concentration-dependent manner and extract showed cell growth inhibition against colon and liver cancer (figure-1). Therefore, *T. arjuna* plant containing these bioactive compounds may serve as a potential source in the treatment of colon & liver cancers. Many plant extract destroy the cancer cell lines through activating apoptosis or through effecting growth regulators. The activity might be depended upon the type of cancer cell lines and mechanism of action of the plant extract. The effect of an Ayurvedic formulation of *T. arjuna*, known as 'Arjuna Kwatha' was assessed by Rao *et al* [11]. Plant extract containing these bioactive organic compounds may be a good potential source for its possible use in the treatment of colon and liver cancers.

4. Table and figure

Table No 1: Showing the presence of Bioactive Compounds in *T. arjuna* bark extract.

Active Compounds→	protein	alkaloids	Terpenoids	phenolics	flavonoids	Tannin
Present (+) / Absent (-)→	+	+	+	+	+	+
	Present (+)	Absent (-)				

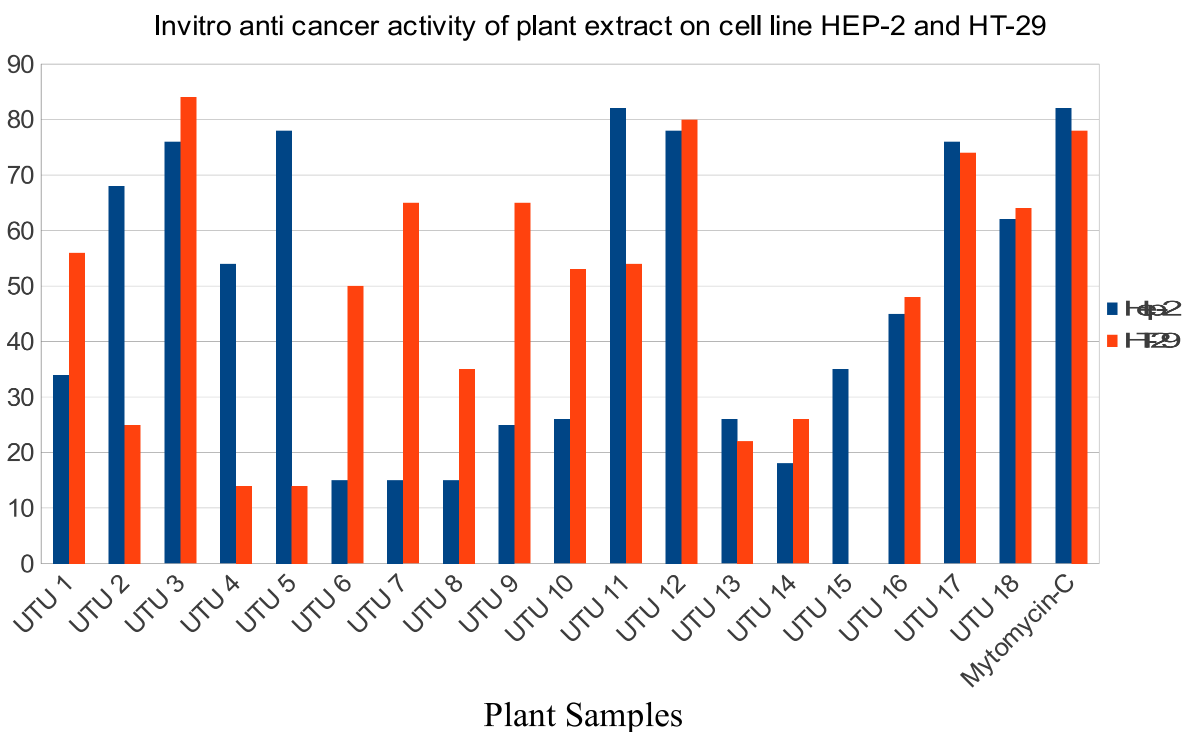

Figure 1: Showing the anti-cancer activity of *T. arjuna* bark extract

5. Conclusion

The anticancer activity might be depended upon the physical morphology of cancer cell lines and mechanism of action of the plant extract. Many plant extract kill cancer cell lines through activating apoptosis and effecting growth regulators. However, based on the published studies flavonoids, alkaloids phenolics, and terpenoids etc seem to be most likely bioactive compounds that showed *in vitro* anti-cancer effect. It was reported that certain products from plants can induce apoptosis in neoplastic cells but not in normal cells [5]. There are large number of recognized inhibitors of mutagens/carcinogens are basically of plant origin and have high diverse chemical nature [14]. Cancer therapy in the form of surgery or radiotherapy is effective when the disease is detected in early stages but many cancers are diagnosed when a primary tumour have metastasized to other parts of the body. The main form of treatment at this point is chemotherapy [8]. Denny and Wansbrough [3] reported that a major challenge is to design new drugs that will be more effective for cancer cells and have lesser side effects. The current research work may provide clue for further research in characterizing the role of pure constituents against cancer cell lines.

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Conflict of Interests:

Authors declare that there is no conflict of interests in respect to the publication of this research paper.

References

[1] Cancer Research U.K. and International Agency for Research on Cancer, Cancer stats Cancer Worldwide, (2011).

[2] Davis, C. D. and Milner, J. A., Diet, Physical Activity and Cancer Prevention. Nutrition Guide for Physicians, 379- 393, (2010) .

[3] Denny, B. and Wansbrough, H). The design and development of anti-cancer drugs. XII Biotech J Cancer Drugs; 1-12, (1995).

[4] Dhiman A.K. ,Ayurvedic Drug Plants, Daya Publishing 7th Edition, New Delhi, (2006).

[5] Hirano T., Abe K., Gotoh M., Oka K., Citrus flavone tangeretin inhibits leukaemic HL-60 cell growth partially through induction of apoptosis with less cytotoxicity on normal lymphocytes. Br J Cancer, 72:1380–1388, House; Pp(41-43), (1995).

[6] Jagan Rao N, Subash KR, Sandeep Kumar K. Role of phytotherapy in gingivitis: A review. Int J Pharmacol, 8:1-5, (2012).

[7] Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ: Cancer statistics, C.A.Cancer J Clin- 58:71–96, (2008).

[8] Liu, W. J. H. Traditional Herbal Medicine Research Methods: Identification, Analysis,Bioassay, and Pharmaceutical and Clinical Studies, John Wiley & Sons, 1-26, (2011).

[9] Martin-Cordero, C.; Leon-Gonzalez, A. J.; Calderon-Montano, J. M.; Burgos-Moron, E.And Lopez-Lazaro, M.. Pro-oxidant natural products as anticancer agents, current drug targets, 13(8): 1006-1028, (2012).

[10] Monks. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines, J Natl.Cancer Institute, 83 757-766, (1991).

[11] Pandey, M. Debnath, M., Gupta, S. and Chikara, S. K., Phytomedicine: An ancient approach turning into future potential source of therapeutics. J. Pharmacog. Phytother.3 : 27-37, (2011).

[12] Prachayasittikul S, Saraban P, Cherdtrakulkiat R, Ruchirawat S, Prachayasittikul V. New bioactive triterpenoids and antimalarial activity of Diospyros rubra Lec. EXCLI J9:1, (2010a).

[13] Rao BCS, Singh RH, Tripathi K. Effect of Terminalia arjuna (W&A) on regression of LVH in hypertensives: a clinical study. J Res Ayurveda Siddha. 22: 216e227, (2001).

[14] Sawadogo, W. R.; Schumacher, M.; Teiten, M. H.; Dicato, M. and Diederich, M.Traditional West African, pharmacopoeia,

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plants and derived compounds for cancer therapy. Biochem. Pharmacol, 84: 1225-40, (2012).

[15] Skehan, P. New colorimetric cytotoxicity assay for anticancer drug screening, Journal of National Cancer Institute, 82: 1107–1112,(1990).

[16] Tiwari K.L., Jadhav S.K., Joshi V., An updated review on medicinal herb genus Spilanthes, Chin J Integr Med, 9: 1170-8, (2011).

[17] Verma S.K., Singh S. K., Singh S.S. and Mathur A., In vitro cytotoxicity of Cannabis sativa and Trigonella foenum graecum against human cancer cell lines, Journal of Chemical and Pharmaceutical Research, 2 (4):861-865, (2010).

[18] Wall M.E,.,Wani M.C, Hughes T. J.,& Taylor H. Plant antimutagenic agents 1,General bioassay and isolation procedures, J Nat Prod, 51, 866, (1995).