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ANTICANCER ACTIVITY OF *TERMINALIA ARJUNA* AGAINST HUMAN CANCER LINE CELL

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ABSTRACT: *In-vitro* cytotoxicity testing has become an integral aspect of drug discovery because it is a convenient, cost-effective, and predictive means of characterizing the toxic potential of new chemical entities. The early and routine implementation of this testing is a testament to its prognostic importance for humans. Anticancer activity of different concentrations (20-100) of plant extracts *Terminalia arjuna* was shown against Hep3-B. MTT [(3, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide)] assay was done *in-vitro* cytotoxicity test. The low concentration does not show the activities were as extract with 80 µl, and 100 µl concentration showed the activity against Human hepatoma cell line.

Keywords: *Terminalia arjuna*, MTT, *In-vitro* cytotoxicity

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INTRODUCTION: A large number of plants and their isolated constituents have been shown to have potential immunity. Some medicinal plants have been shown to exert anti-inflammatory, anti-stress and anti-cancer effects by modulating the immune functions [1]. In designing a search for novel prototype antifungals, higher plants are a logical choice, because secondary metabolites are widely distributed among higher plants [3]. But only a few have been evaluated for their activity against human, animal, and plant pathogenic fungi. The majority of clinically used antifungals have various drawbacks in terms of toxicity, efficacy as well as cost.

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Their frequent use has also led to the emergence of resistant strains. Concerns have been raised about both the environmental impact and the potential risk related to the use of synthetic fungicides [6]. Therefore there is a need to search for plants of medicinal value. The species of Terminalia are very well known for their therapeutic values and are useful as anticancer, antigenotoxic, anti-inflammatory, anti-HIV, antidiabetic and hepatoprotective activities. The plants used in the present study are *Terminalia catappa* and *Terminalia arjuna* (Combretaceae).

The main objectives of this study are to examine the antifungal property of ethanolic and methanolic leaf extracts of *Terminalia catappa* and *Terminalia arjuna*. *Terminalia arjuna* is a tree of Combretaceae family which also known as 'Arjuna' or 'Arjun.' This tree is usually grownup to 25-meter height and found throughout India and some regions of Bangladesh [5]. *Terminalia arjuna* is the only herb that helps uphold a healthy heart and

decrease the effects of stress and anxiety. The bark of Arjuna is useful as an anti-ischemic and cardioprotective agent in hypertension (High blood pressure) and in ischemic heart illness (IHD), particularly in uneasy cardiac rhythm, angina or myocardial infarction. The bark powder possesses diuretic and a broad spectrum tonic effect in cases of cirrhosis of the liver, Terminalia may also be used treating hypercholesterolemia by reducing LDL levels [7]. Previously, it was found that the extract of bark contains arjunic acid, arjungenin, and an arjunetin compound which possess strong activity against epidermis [8].

However, very little work is reported with the bioactivity of root, leaves and fruits extract of *T. arjuna* and so far no work has been done on a combinatorial basis like leaf-fruit extract. On this perspective, this work was objected to carrying out to investigate the cytotoxicity and antimicrobial activity of ethanolic leaf-fruit extract of *T. arjuna* against selected Gram-positive and Gram-negative bacteria in Bangladesh. Alter the normal properties of cells. In cancer cells, the normal control systems that prevent cell overgrowth and the invasion of other tissues are disabled.

These altered cells divide and grow in the presence of signals that normally inhibit cell growth; therefore, they no longer require special signals to induce cell growth and division. As these cells grow, they develop new characteristics, including changes in cell structure, decreased cell adhesion, and production of new enzymes. These heritable changes allow the cell and its progeny to divide and grow, even in the presence of normal cells that typically inhibit the growth of nearby cells. Such changes allow cancer cells to spread and invade other tissues. Certain products from plants of *Terminalia arjuna* are known to induce apoptosis in neoplastic cells but not in normal cells [2].

METHODOLOGY:

Selection of Plants: In the present work, *Terminalia arjuna* were screened for its potent anticancer activity.

Preparation of Plant Extracts: Thoroughly washed dried leaves of *Terminalia arjuna* were dried in the shade for one week. Plant parts were placed in an oven at 38 °C and then powdered with mortar and pestle. Plant materials 1 gram/100 ml of solvent were soaked separately in petroleum ether for 72 h. The mixture was stirred after every 24 h using a sterile glass rod. At the end of extraction, the extract was passed through Whatman filter paper no. 1. The filtrates obtained were concentrated in vacuum using rotary evaporator at 30 °C.

(Hep3-B) Cell Maintenance: Human hepatoma cell line (Hep3-B) was obtained from NCL (National chemical laboratory), India. Cells were grown as monolayers in RPMI-1640 medium, supplemented with 10% (v/v) heat-inactivated FBS, antibiotics (penicillin 100U/mL, gentamycin 100µg/mL) and 1mmol/L sodium bicarbonate under standard conditions (37 ºC) in a controlled humidified atmosphere containing 50mL/L CO_2.

***In-vitro* Assay for Cytotoxic Activity:** The anticancer activity was determined by the cytotoxic potential of the test material using human cancer cell lines, which were allowed to grow on tissue culture plates in the presence of test material. The cell growth was measured using ELISA reader after staining with MTT (3-(4, 5 Dimehylthiozol-2-yl) - 2, 5- Diphenyl tetrazolium bromide assay. This binds to basic amino acid residues in the trichloroacetic acid (TCA) fixed cells.

Preparation of Cell Suspension for Assay: Human cancer cell lines were grown in multiple tri conical flasks (TCFs) at 370C in an atmosphere of 5% CO_2 and 90% relative humidity in complete growth medium to obtain enough number of cells. The flasks with cells at the subconfluent stage were selected. Cells were harvested by treatment with Trypsin-EDTA. Cells were separated to single cell suspension by gentle pipetting action, and the viable cells were counted in a hemocytometer using trypan blue. Cell viability at this stage should be >97%. Viable cell density was adjusted to 5,000 - 40,000 cells/100µl depending upon the cell line Monks (1991). 100 µl of cell suspension together with 100µl of complete growth medium was added into each well. The plates were incubated at 37 ºC for 24 h in an atmosphere of 5% CO_2 and 90% relative humidity in a CO_2 incubator. After 24 h, the test material, DMSO (vehicle control), and positive control were added.

Cytotoxicity Determination by MTT Assay: Cancer cells were subcultured in RPMI-1640 media supplemented with 2mM L-glutamine adjusted with 1.5g/L Sodium bicarbonate and 10% fetal calf serum incubated at 37 ºC in 5% CO_2 incubator. Different concentrations of plant extract (20-100µl) were added to the het-3 cancer cells, seeded in 96-well microliter & incubated at 37 ºC for 24 h. At the end of the treatment, 20µl of MTT [(3, 5 - dimethylthiazol-2-yl) - 2, 5-diphenyltetrazolium bromide)] was added to each well & the microliter plate was incubated for 4 h at 37 ºC. Finally, detergent reagent (100 µl) was added to each well, after which optical absorbance was read at 570 nm on multi-well spectrophotometer plate reader.

The percentage of viability was calculated as follows:

Cell Viability = Optical density of samples/Optical density of control × 100

RESULT AND DISCUSSIONS:

Anti-cancer Activity of *Terminalia-arjuna via* MTT Method: Different concentrations of plant extract (20- 100µl/ml) shown in **Table 1**.

TABLE 1: DIFFERENT CONCENTRATIONS OF PLANT EXTRACT

Sample	Control	20 µl/ml	40 µl/ml	60 µl/ml	80 µl/ml	100 µl/ml
Terminalia –arjuna	1.00	0.43	0.54	0.55	0.73	0.77

From the earliest times, herbs have been prized for their pain relieving and healing abilities, and today we still rely largely on the curative properties of plants. According to the World Health Organization, 80% of the people living in rural areas depend on medicinal herbs as the primary healthcare system. The synthetic anticancer remedies are beyond the reach of the common man because of the cost factor. Herbal medicines have a vital role in the prevention and treatment of cancer, and medicinal herbs are commonly available and comparatively economical.

Certain biological response modifiers derived from herbs are known to inhibit the growth of cancer by modulating the activity of specific hormones and enzymes. Some herbs reduce the toxic side effects of chemotherapy and radiotherapy. Scientists all over the world are concentrating on herbal medicines to boost immune cells of the body against cancer.

By understanding the complex synergistic interaction of various constituents of anticancer herbs, the herbal formulations can be designed to attack the cancerous cells without harming normal cells of the body. Medicinal herbs are also a significant source of synthetic and herbal drugs. Plants are a storehouse of good variety of compounds. Latest and previous studies have concluded the beneficial aspects of plant-derived drugs as a good source of anticancer activity agents. The test sample showing growth inhibition more than 70% at 100µg/ml is considered to be active. In the present study, we conclude that the higher plant concentration like (80µl/ml) and (100µl/ml) showed the anticancer activity were as the lesser concentration does not show the anticancer activity against human hepatoma cell line (Hep_3-B).

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REFERENCES:

1. Mathur A, Verma SK, Bhat R, Singh SK, Prakash A, Prasad GBKS and Dua VK: Journal of Chemical and Pharmaceutical Research 2010, 2(4): 264-70.
2. Bhattacharya A, Ghosal S and Bhattacharya SK: Anti-oxidant effect of Withania somnifera glycol withanolides in chronic foots hock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. J Ethnopharmacol 2001; 74: 1-6
3. Caceres A, Jauregui E, Herrera D and Logemann H: Plants used in Guatemala for the treatment of dermatomucosal infections: Screening of 38 plants extracts for anticandidal activity. J Ethanopharmacol 1991; 33: 277-83.
4. Dwivedi S and Udupa N: *Terminalia arjuna*: pharmacognosy, phytochemistry, pharmacology and clinical use. Fitoterpia 1989; 60: 413-20.
5. Khulbe K and Sati SC: Antibacterial activity of *Boenninghausenia albi* flora Reichb. (Rutaceae). Afr J Biotechnol 2009; 8: 6346-48.
6. Miller AL: Botanical influences on cardiovascular disease. Altern Med Rev 1998; 3(6): 422-31.
7. Singh DV, Gupta MM, Kumar TRS, Saikia D and Khanuja SPS: Antibacterial principles from the bark of *Terminalia arjuna*. Curr Sci 2008; 94(1): 27-29.
8. Verma SK, Singh SK, Singh 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 2010, 2(4): 861-65.

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