

BIBLIOGRAPHIC INFORMATION SYSTEM

Journal Full Title: Journal of Biomedical Research & Environmental Sciences

Journal NLM Abbreviation: J Biomed Res Environ Sci

Journal Website Link: <https://www.jelsciences.com>

Journal ISSN: 2766-2276

Category: Multidisciplinary

Subject Areas: Medicine Group, Biology Group, General, Environmental Sciences

Topics Summation: 128

Issue Regularity: Monthly

Review Process type: Double Blind

Time to Publication: 7-14 Days

Indexing catalog: [Visit here](#)

Publication fee catalog: [Visit here](#)

DOI: 10.37871 ([CrossRef](#))

Plagiarism detection software: [iThenticate](#)

Managing entity: USA

Language: English

Research work collecting capability: Worldwide

Organized by: [SciRes Literature LLC](#)

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ORIGINAL RESEARCH ARTICLE

Phytochemical Analysis and Anticancer Activity of *Azadirachta Indica* Ethanolic Extract against A549 Human Lung Cancer Cell Line

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ABSTRACT

Azadirachta indica phytochemicals are found to have against malignant growth and hostile to bacterial properties. In the specific examination, the coupling proficiency of five mixes that are available in the *Azadirachta indica* with all the eleven proteins through *in silico* techniques was completed. Plant removes ensure against harmful compound instigated injury by expanding the body's degrees of cell reinforcement particles, for example, glutathione, and improving the action of cancer prevention agent chemicals. A549 cells treated with *Azadirachta indica* ethanolic separate in various hours (6, 12, 24 and 36 hours) after the 36 hours the cells development are controlled. As there are re-established interests in home grown based meds to hinder the results of manufactured medications, *Azadirachta Indica* L. a leaf contains phytochemical intensifies having all the more free revolutionary rummaging just as anticancer exercises.

INTRODUCTION

Natural antibiotic properties of plant secondary metabolites

A local herb known as neem (semambu) or its logical name *Azadirachta indica* has been utilized widely in customary treatment because of its restorative properties. Neem leaves have been utilized generally for treating a few epidermal dysfunctions, for example, skin inflammation, psoriasis, and skin break out [1]. In the particular assessment, the coupling viability of five blends that are accessible in the *Azadirachta indica* with all the eleven proteins through *in silico* procedures was finished. Gas Chromatography (GC) and Mass Spectrometry (MS) make a successful blend for synthetic investigation. Gas Chromatography (GC) is perhaps the most broadly utilized methods [2-5]. Thus, an endeavor has been made to assess the productivity of different natural solvents, antibacterial movement and distinguish the substance [6,7]. Segments by GC-MS investigation of rough bark separates from the *Azadirachta indica* against those bacterial strains [8-11]. Neem is wealthy in cancer prevention agents and assists with boosting invulnerable reaction in tissues of influenced skin region [12-17]. It likewise comprises of bioactive mixes for antibacterial, antifungal, and anticancer exercises [18]. In this investigation, neem leaves remove was utilized in creating natural neem cleanser as a solution for restoring skin issues (Figure 1).

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DOI: 10.37871/jbres1225

Submitted: 11 March 2021

Accepted: 15 April 2021

Published: 16 April 2021

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OPEN ACCESS

Subject: Medicine Group

Topic & Subtopic(s): Pharmacology; Cancer

Keywords:

- *Azadirachta indica*
- Human cancer cell line A549
- Phytomedicine

VOLUME: 2 ISSUE: 4





Figure 1 *Azadirachta indica* medicinal values.

Phytochemicals shows antioxidant activities

The free revolutionary present during our body are responsible for the age of numerous illnesses. One can keep himself from the illnesses create by activity of free extremists simply by deactivation of free revolutionary [19]. Cancer prevention agents have property to stop the movement of free extremists before its activity to words the organic cell of human body [20-23]. It has been accounted for that the oil ether, methanol and watery concentrates of the leaves of *Azadirachta indica* were screened for their enemy of microbial movement utilizing the cup plate agar dissemination technique [24,25]. They were tried against six microscopic organisms; two Grampositive microorganisms (*Bacillus subtilis* and *Staphylococcus aureus*) and four Gram-negative microbes (*Escherichia coli*, *Proteusvulgaris*, *Pseudomonas aeruginosa* and *Salmonella typhi*) [26-29]. The powerlessness of the microorganisms to the concentrates of this plant was contrasted and one another and with those anti-infection agents [30]. The methanol concentrate of *Azadirachta indica* showed articulated movement against *Bacillus subtilis* (28mm). *Azadirachta indica* has a place with the family Meliaceae generally called as Neem (English), Vempu (Tamil) and is found all through India [31]. A medium to enormous estimated tree, grow up to 50 feet (15 m) tall and live for 200 y it has been recommended that ethanol extricates from plants utilized.

Activity against malaria

The antimalarial movements of the tablet suspension of the bark and leaf of *Azadirachta indica* were assessed. On *Plasmodium yoelli nigeriensis* contaminated mice [32]. "The tablet suspensions showed high prophylactic, moderate suppressive and a negligible therapeutic schizonticidal impact [33]. The tablet suspensions from the leaf and bark at a grouping of 800 mg/kg and chloroquine at a centralization of 62.5 mg/kg body weight delivered normal rate (%) parasitaemia of 79.6%, 68.2% and 99.5% for leaf, bark and chloroquine, individually, in chemosuppression. Likewise in the prophylactic treatment, the tablet suspensions at 800 mg/kg and pyrimethamine at a convergence of 0.35 mg/kg

gave a normal parasitaemia decrease of 75.3%, 65.6% and 98.3% for the leaf, bark and pyrimethamine, individually [29]. Convenient was sign of reasonable valuable impact. In the only remaining century, huge advancement in bio-clinical science has vanquished numerous sicknesses anyway disease stays vague particularly from a restorative viewpoint [34]. Malignancy is as yet the most widely recognized reason for death from sickness after myocardial dead tissue. Tumor or neoplasm is generally characterized as a development of an irregular mass of tissue because of uncontrolled cell development, while disease is the term of every single dangerous tumor [27]. Numerous logical investigations that zeroed in on the pharmacological action of bio-dynamic parts from plants lately increment the premium from academic local area to distinguish some novel diseases suppressant. Natural focuses of phytochemicals in mammalian cells were found to metastases [22]. Also, epidemiological examinations recommend that the day by day admission of specific phytochemicals can lessen the frequency of a few sorts of malignant growths [17]. The primary framework in the body for breathing is respiratory framework. The respiratory framework incorporates the nose, mouth, (windpipe) and aviation routes to every lung. The aviation routes to every lung are called huge aviation routes (bronchi) and little aviation routes (bronchioles). Cellular breakdown in the lungs is the second most regular disease in the two people on the planet. It represents about 14% of all new diseases in the two people. For smokers the danger of cellular breakdown in the lungs is a lot higher than in non-smokers [11]. Cellular breakdown in the lungs is a malignancy that begins in the lungs. Cellular breakdowns in the lungs are thought to create over numerous years. There are 2 significant sorts of cellular breakdown in the lungs. On the off chance that a cellular breakdown in the lungs has qualities of the two sorts it is known as a blended little cell/huge cell malignancy this isn't normal. Uranium is principle malignancy causing specialist found in rocks and soil [13]. As it rots, a gas called radon is freed and gets blended into air and water. Diggers of uranium have high danger of creating cellular breakdown in the lungs. A few investigations of radon found in the home have indicated

its impact in causing cellular breakdown in the lungs, while different examinations have not. The danger for cellular breakdown in the lungs may rely upon how much level of radon gets presented to a person. The plant fabricated materials are appointed fundamental or discretionary metabolites [19]. Fundamental metabolites are extensively passed on in nature, occurring in some design in essentially all living creatures. In higher plants such blends are as often as possible stuffed in seeds and vegetative amassing organs and are needed for physiological headway considering their part in fundamental cell assimilation. Fundamental metabolites got from higher plants for business use are high volume-low worth mass manufactured mixes (for instance vegetable oils, unsaturated fats, starches, etc).

MATERIAL AND METHODS

Plant collection

The fresh leaves of *Azadirachta indica* were collected from Saliyamangalam, Thanjavur District, Tamil Nadu, India (Figures 2 & 3).



Figure 2 *Azadirachta indica*.

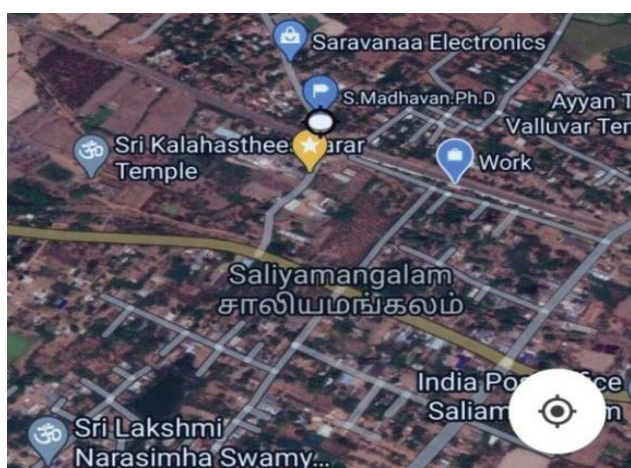


Figure 3 Map 1; study area.

Plant material

The *Azadirachta indica* leaves was dried up under shade, specifically diminish to a decently crude powder, and put away in golden hued sealed shut holders. The crude type of the medication was utilize for the declaration of physicochemical boundaries similar to dampness content, debris esteems, expanding file, frothing record, unfamiliar natural issue, extractive qualities, and fluorescence analysis.

Phytochemical studies

Secondary metabolites in the present studies were carried out on the plant sample revealed the presence of medicinally active constituents. Beneficial drugs and to improve the patient health.

Preparation of extracts

The powdered plant samples of leaves (100 g) were used for successive solvent extraction (500 ml) with increasing order of polarities like ethanol, methanol. At that point it is kept in an orbital shaker at 190–220 rpm for 48 hours. The supernatant was collected, filtered through Whatman No.1 filter paper and the extract were concentrated by a Rotary flask evaporator at a specific temperature was used based on the solvent system. Each time previous to extract through the next solvent the remains was dried thoroughly to remove the solvent used. The acquired dried concentrate was then precisely gauged, put away in little vials at -20°C and utilized for the accompanying examinations.

Phytochemical screening

The preliminary phytochemical evaluation was accepted out by using standard procedure [8,9].

Culturing of cell lines

The Vero and human lung cancer cells (A549) were obtained from Kings Institute of Preventive Medicine and Research, Guindy, Chennai. The cells were grown in 96-well Tissue Culture (TC) plate in Dulbecco's Minimum Essential Medium (MEM) with Trypsin-Phosphate-Verseneglucose (TPVG) solution, 10% New Born Calf Serum (NBCS) (Gibco-Invitrogen), 100 U/mL of penicillin (Gibco-Invitrogen) and 100 $\mu\text{g}/\text{mL}$ of streptomycin (Gibco-Invitrogen). The cells were incubated in CO_2 incubator (Haier Electric Co., Ltd.) at 37°C in 95% humidified atmosphere enriched by 5% CO_2 and sub-cultured every 3–4 days once.

MTT cell viability assays

The MTT assay was done using the methodology of monolayer of cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using growth medium. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells /well) was added. After 24 hours, when a partial monolayer was

formed, the supernatant was flicked off, the monolayer was washed once and 100 µl of leaves of *Azadirachta indica* leaves ethanolic extract with different concentrations (100, 200, 250, 500 and 1000 µg/ml) was added to each well. The plates were then incubated at 37°C for 3 days in 5% CO₂ atmosphere, and microscopically examined at the end of 6, 12, 24 and 36 hours for recording the result. After 72 hours, the test solutions in the wells were discarded and 50 µl of MTT in HBSS-PR was added to each well. The plates were gently shaken and incubated for 3 hours at 37°C in 5% CO₂ atmosphere. The supernatant was removed and 50 µl of propanol was added and therefore the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at the wavelength of 540 nm. The percentage growth inhibition was calculated using the formula given below:

$$\% \text{ Cytotoxicity} = \frac{1 - \text{Abs test}}{\text{Abs Control}} \times 100$$

Statistical analysis

Statistical analysis was performed by one way Analysis of Variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT) using Software Package for the Social Science (SPSS) software package version 15.00. Results were expressed as Mean ± Standard Deviation for p values <0.05 were considered significant for analysis of percent inhibition of cell growth.

RESULTS AND DISCUSSION

The plants and its derivatives may considered as good sources of natural phytochemicals for medicinal uses such as against cancer, diabetic mellitus, cardiovascular diseases, aging and other diseases related to radical mechanisms. Plant-derived phytochemical therapy may be helpful for various free radical mediated diseases. Determination of the natural phytochemicals and antimicrobial compounds will help to develop new drugs [35-37]. The result of the preliminary phytochemical analysis of this present study may give credence to its ethno medicinal tradition [18]. In medicine, it is used in hypercholesterolemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory, and weight loss among others. It is also known to contain antimicrobial properties.

Preliminary phytochemical screening

India is most likely the greatest creator of restorative flavours on the planet [12]. While the mechanism of action possessed by tannins is by disrupting the worm’s negative ion body charge into positive ions (protonization), which then attract positive worm body proteins in the gastrointestinal tract, thus disrupting the metabolism and homeostasis of the worm’s body and required into secondary metabolites (Table 1).

Table 1: Qualitative analysis of Phytochemicals analysis *Azadirachta indica* leaves extract.

S. No	Analysed Phytochemicals factor	Ethanol	Methanol
	Tannin	++	+
	Phlobatannins	-	+
	Saponin	+	+
	Flavonoids	++	+
	Steroids	++	-
	Terpenoids	+	+
	Triterpenoids	+	+
	Alkaloids	++	+
	Carbohydrate	+	-
	Protein	++	-
	Anthraquinone	-	-
	Polyphenol	++	+
	Glycoside	+	-

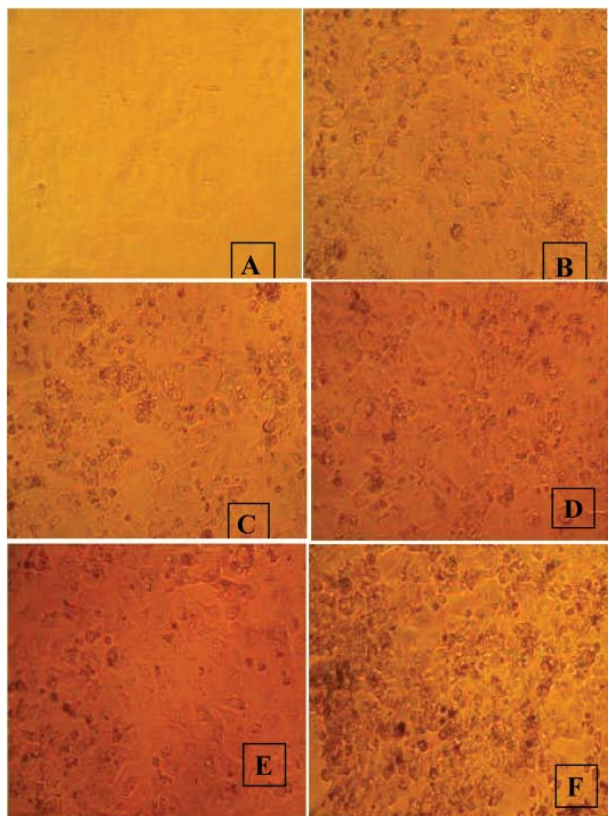
Indications: “+” means positive activity, “-” means negative activity.

A natural cleanser has been delivered effectively from neem leaves separate in this examination. The outcomes from the physicochemical properties of the neem cleanser arranged were contrasted with neem seed oil cleanser and business neem cleanser. The outcomes suggest that the neem cleanser created is reasonable for human skin. In addition, it is an item advancement of a characteristic sedated cleanser created from neem leaves remove that is liberated from synthetic substances, for example, Sodium Sulfate (SLS), fake colorant, and fake scent, hence can be a reasonable elective treatment for buyers who have skin issues [15]. However, there are numerous reports on the *in vitro* investigation of these mixes and its restorative and harmful properties, there are no *in silico* examines that foresee the authoritative and dynamic districts particularly with these proteins [6]. Our investigation is an endeavour to anticipate the coupling site and the coupling deposits. Nonetheless, approval of our outcomes through *in vivo* and *in vitro* tests and furthermore with creature models will edify trust for the future improvement of additional powerful medications for the treating of pharmacological significance of contain the properties, for example, antibacterial enemy of diabetic and pain relieving.

Anticancer activity on A549 cell lines

The *Azadirachta indica* ethanolic extract showed the anticancer activity against A549 cell line in a dose dependant manner. A549 cells treated with *Azadirachta indica* ethanolic extract in different hours (6, 12, 24 and 36 hours) after the 36 hours the cells growth are controlled. The *Azadirachta indica* ethanolic extract showed a maximum inhibition of 68% at 500 µg/ml against A549 lung cancer cells [11]. The present investigation the minimum inhibition of 12% was recorded at 100 µg/ml concentration. A549 cell normally contain a polygonal shape and expanse similar [9]. The *Azadirachta*

indica ethanolic extract showed a maximum inhibition of 68% at 500 µg/ml against A549 lung cancer cells. The present investigation the minimum inhibition of 12% was recorded at 50 µg/ml concentration. Herbal mediated silver nanoparticles playing important role to create recyclable, cost effective and stable nanoparticles [12]. The investigates on union of silver nanoparticles utilizing different plant removes found that it is supplementary secure and better in malignant growth treatment, however more plants are as yet not investigated for the combination of nanoparticles and its applications of ethanolic commercial drug in cell restore 1000 µg/ml businesses.



Cell line image: A: Control; B: 100 µg/ml; C: 200 µg/ml; D: 250 µg/ml; E: 500 µg/ml; F: 1000 µg/ml

The cells of living beings can be considered as miniature synthetic reactors creating huge number of substance mixes through metabolic responses. Ayurvedic arrangement of medication in India and is utilized in relieving different sicknesses like stomach issues, gastric ulcers and to animate craving [14]. The cytotoxic properties were assessed, on Malignant growth cells of Breast cancer (MCF-7), cellular breakdown in the lungs (A-549) cell lines and contrast and ordinary cell line L-6 (Rat, Normal muscle) utilizing MTT colorimetric test for 24, 48 and 72 hrs. There is no orderly work that has been embraced on this plant and this is the principal report of the phytochemical screening, cell reinforcement and anticancer exercises evoked by saponin from *Decalepis hamiltonii* [14]. Combination of home grown intervened silver nanoparticles gives controlled and

focusing on activity of medication, which can likewise defeat the issues related with traditional disease medicines [38]. The current investigation has been detailed to comprehend the phytochemical screening, in vitro cell reinforcement and anticancer properties evoked by saponin from foundations of *Decalepis hamiltonii*.

CONCLUSION

Allopathic medicine is potential sources of antiviral, antitumor and antimicrobial agents. The plausible instrument in cause the phone demise or apoptosis is by interfacing with the phone film proteins and making the phone release its constituents lastly driving passing or possibly it can connect with the DNA or cell declining pathways and controlling the pathways driving or scenery off the cell demise pathways. The presence of such assortment of phytochemicals might be credited to the therapeutic qualities of this plant *Azadirachta indica*. The consequences of this investigation uphold the conventional utilization of *Azadirachta indica* bark as an antibacterial specialist. In a few reports, these mixes can be answerable for the preventative properties credited to this plant in famous and conventional medication.

ACKNOWLEDGEMENT

The author is also very grateful to the Secretary and Correspondent and the Principal of A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613 503, Thanjavur (Dt), for providing the excellent infrastructure and necessary facilities to carry out my research work successfully.

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How to cite this article: Azhagu MS. Phytochemical Analysis and Anticancer Activity of *Azadirachta Indica* Ethanolic Extract against A549 Human Lung Cancer Cell Line. *J Biomed Res Environ Sci*. 2021 Apr 16; 2(4): 280-285. doi: 10.37871/jbres1225, Article ID: JBRES1225