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EVALUATION OF ANTICANCER POTENTIAL OF ETHANOLIC EXTRACT OF *ACACIA FARNESIANA* BARK

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ABSTRACT

Cancer remains one of the leading causes of mortality worldwide, necessitating the search for safer and more effective therapeutic agents from natural sources. The present study aimed to evaluate the in vitro anticancer potential of the ethanolic bark extract of *Acacia farnesiana* against human breast cancer (MCF-7) and cervical cancer (HeLa) cell lines. Bark samples were collected, authenticated, shade-dried, and extracted using ethanol through Soxhlet extraction. The cytotoxic activity of the extract was assessed using the MTT assay at concentrations ranging from 1 to 100 µg/mL. Results demonstrated a concentration-dependent reduction in cell viability in both cancer cell lines. At the highest tested concentration (100 µg/mL), cell viability decreased to 28.4% in HeLa cells and 19.4% in MCF-7 cells, indicating significant antiproliferative activity. The MCF-7 cell line exhibited greater sensitivity to the extract compared to HeLa cells. The observed cytotoxic effects may be attributed to the presence of bioactive phytoconstituents such as flavonoids, phenolic compounds, and tannins, which are known to induce apoptosis and inhibit cancer cell proliferation. These findings suggest that the ethanolic bark extract of *Acacia farnesiana* possesses promising anticancer potential and may serve as a valuable source of lead molecules for the development of novel plant-based anticancer therapeutics. Further mechanistic studies and in vivo investigations are warranted to validate its efficacy and safety.

Keywords: Breast cancer, Cervical cancer, MTT assay, *Acacia farnesiana*, etc.

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INTRODUCTION

The study by Sathishkumar et al. revealed that 1,461,427 new cases of cancer were expected in India in 2022, with a crude rate of 100.4 cases per 100,000 people. The statistics are shocking: 1 in every 9 persons in India are at risk of developing cancer at some point in their life. Interestingly, lung cancer was the most common type of cancer in men and breast cancer was the most common type of cancer in women. These estimates can also be used to help design specific, preventive and control initiatives. Such interventions may include early detection interventions, interventions to reduce risk factors, and effective interventions for managing the condition [1]. There is a large amount of research into finding more effective treatments that have fewer side effects. The key is on developing therapies that are innovative enough to specifically target and kill malignant tumor cells without affecting normal ones. This research is in line with the ultimate goal of cancer chemotherapy in which selective drugs can kill or transform cancerous cells into non-cancerous states [2]. One practical solution to this is to look at traditional herbs and see if they can be used as a source for developing a drug. Traditional medicine systems have heavily relied on plants, playing a pivotal role in their evolution. Many metabolites and phytochemicals have a wide variety of pharmacological effects in humans. This encompasses but is not restricted to flavonoids, phenolics, alkaloids, tannins, gums, oils, glycosides and resins. These bioactive components are responsible for the various medicinal properties found in medicinal plants [1, 3]. Herbal medicines also have a unique advantage in that they are easily accessible to consumers, not only in pharmacies, but in many other retail outlets as well. Herbal medicine is used by a large percentage of people in the world, predominantly Asian and African countries, about 80% [2] [4, 5]. Plants are the source of many drugs that are essential to modern medicine [6].

Acacia farnesiana is widely distributed across different regions and is known by various vernacular names in different languages, reflecting its extensive traditional use and cultural significance. In Bengali, it is commonly referred to as Guva babla, Guva babul, or Guya bebula. In Assamese, it is known as Tarua kadam. *Acacia farnesiana* has been widely used in traditional systems of medicine, where different parts of the plant are utilized for the treatment of various ailments [7,8]. The bark is traditionally employed as an astringent and demulcent, particularly for managing bleeding gums, cough, typhoid, swelling of neck glands, dysentery, and stomach disorders [9]. It is also reported to possess anthelminthic, antimalarial, and anti-rheumatic properties. Its flowers are used as an emetic, antispasmodic, and stimulant, and are traditionally indicated for stomach aches, dyspepsia, and headaches. Additionally, the flowers are valued in the preparation of cassie perfume. The roots are extensively used in folk medicine for conditions

such as tuberculosis, throat infections, diarrhoea, snakebite envenomation, fever, and rheumatism. The increasing evidence demonstrates the multi-faceted therapeutic potential of *A. farnesiana* and its role in numerous studies. The well-recognized importance of flavonoids in cancer prevention underscores their huge potential in cancer chemoprevention and chemotherapeutic applications [3, 10]. Several flavonoids such as flavanones, daidzein, genistein, quercetin and luteolin have been shown to be effective against a variety of cancers in humans. For example, they have proven to be effective against breast cancer [11, 12] and also against lung cancer [13, 14]. Furthermore, quercetin, luteolin, catechin, epicatechin, myricetin, kaempferol, genistein, apigenin and silymarin have been seen to have considerable effects against human prostate cancer [4, 15,16]. In light of this background, the primary aim of the present study was to investigate anticancer potential of ethanolic extracts in a breast and cervical cancer cell lines.

MATERIALS AND METHODS

Collection and Authentication of Bark

Acacia farnesiana bark were gathered from Alwar, Rajasthan. The identification, authentication of plant was conducted Senior Scientist at the Himachal Pradesh State Biodiversity Board, Shimla, Himachal Pradesh, India.

Preparation of Extract

Freshly collected *Acacia farnesiana* bark was cleaned efficiently under running faucet water and distilled water. It was shade dried at 37ºC to avoid any chemical changes and pulverized into fine powder with appropriate size. This powder was used for extraction of crude drug in ethanol by Soxhlet extraction method. After completion of extraction process, the solvent was carefully removed by distillation and the concentrated extract was obtained after drying under reduced pressure with the help of a rotary evaporator.

Cell lines

Breast and cervical cancer cell lines HeLa (ATCC CCL-2) and MCF-7 (ATCC HTB-22) were sourced from the National Centre for Cell Sciences, Pune. These cell lines were cultivated in a culture medium consisting of minimum essential media (MEM) with Earle's salt, devoid of glutamine, and supplemented with 10% heat-treated fetal bovine serum, 1% of 2 mM L-glutamine, 50 IU/ml penicillin, 50 µg/ml streptomycin, and 1% non-essential amino acids. They were maintained at a constant temperature of 37°C in an environment with 5% CO_2 and 95% humidity. To facilitate consistent growth throughout the experimental duration and to ensure a proportional relationship between the absorbance at 492 nm and the number of cells, the optimal plating density for the both cancer cell lines was established at 3 × 103 cells per well. This

determination was made using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [17].

Antiproliferative MTT assay

The ethanolic extract was evaluated for possible in-vitro anticancer activity against the human breast cancer cell lines HeLa and MCF-7. The effect ethanolic extract of *A. farnesiana* bark (Rajasthan Sample) on MCF-7 and HeLa cell lines viability was evaluated by means of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The method was conducted as previously described. Briefly, 1 × 104 cells/well were seeded in 96-well culture plates and allowed to adhere overnight. On the second day, the cells were stimulated with different concentrations of bark ethanolic extract (0, 1, 5, 10, 20, 50, 75, and 100 µg/mL) and were incubated for 72 hrs. The Control group is represented by cells treated with the solvent DMSO (Sigma-Aldrich). After the 72 hrs incubation period, the cells were treated with 10 µL of 5 mg/mL MTT solution from the MTT kit (Sigma-Aldrich) and incubated for an additional 3 h. The obtained formazan crystals were dissolved in 100 µL of lysis solution provided in the MTT kit. The absorbance was determined at 570 nm with a microplate reader (BioRad, xMark Microplate Spectrophotometer).

RESULTS AND DISCUSSION

The in-vitro cytotoxic effect of the ethanolic bark extract of *Acacia farnesiana* against HeLa (cervical cancer) and MCF-7 (breast cancer) cell lines, evaluated using the MTT assay at concentrations ranging from 1 to 100 µg/mL.

At 0 µg/mL, both cell lines exhibited 100% cell viability, confirming the absence of solvent-induced cytotoxicity. With increasing concentration of the bark extract, a progressive decrease in percentage cell viability was observed in both HeLa and MCF-7 cells, indicating a dose-dependent cytotoxic response.

In HeLa cells, cell viability decreased from 87.6% at 1 µg/mL to 28.4% at 100 µg/mL, whereas in MCF-7 cells, viability declined from 97.8% to 19.4% over the same concentration range. The relatively low standard deviation (SD) values across concentrations indicate good reproducibility and reliability of the experimental data.

The ethanolic bark extract of *Acacia farnesiana* exhibited significant concentration-dependent cytotoxicity against both HeLa and MCF-7 cell lines, as demonstrated by the MTT assay results. The gradual reduction in cell viability with increasing extract concentration confirms the ability of the extract to inhibit cancer cell proliferation.

Comparatively, the MCF-7 cell line showed higher sensitivity to the bark extract than HeLa cells, particularly at higher concentrations. At 50 µg/mL, MCF-7 cell viability was reduced to

36.3%, compared to 44.7% in HeLa cells, and at 100 µg/mL, viability further declined to 19.4% in MCF-7 cells versus 28.4% in HeLa cells. This suggests a cell-line-specific response, possibly due to differences in receptor expression, metabolic pathways or susceptibility to phytochemicals present in the extract.

The observed cytotoxic activity may be attributed to the presence of phenolic compounds, flavonoids, tannins and other bioactive constituents, as confirmed by phytochemical screening, quantitative estimation and FTIR analysis. These compounds are known to induce apoptosis, disrupt mitochondrial function and inhibit cell cycle progression in cancer cells, thereby reducing cell viability.

The dose-dependent cytotoxic pattern observed in this study aligns well with the results of antioxidant assays and molecular docking studies, suggesting that the bioactive compounds present in the bark extract may exert anticancer effects through oxidative stress modulation and interaction with key molecular targets such as estrogen receptor alpha.

Overall, the results demonstrate that the ethanolic bark extract of *Acacia farnesiana* possesses promising in-vitro anticancer activity, with greater efficacy against MCF-7 breast cancer cells. Although the extract did not show complete cytotoxicity at the tested concentrations, the significant reduction in cell viability highlights its potential as a source of lead compounds for further anticancer drug development.

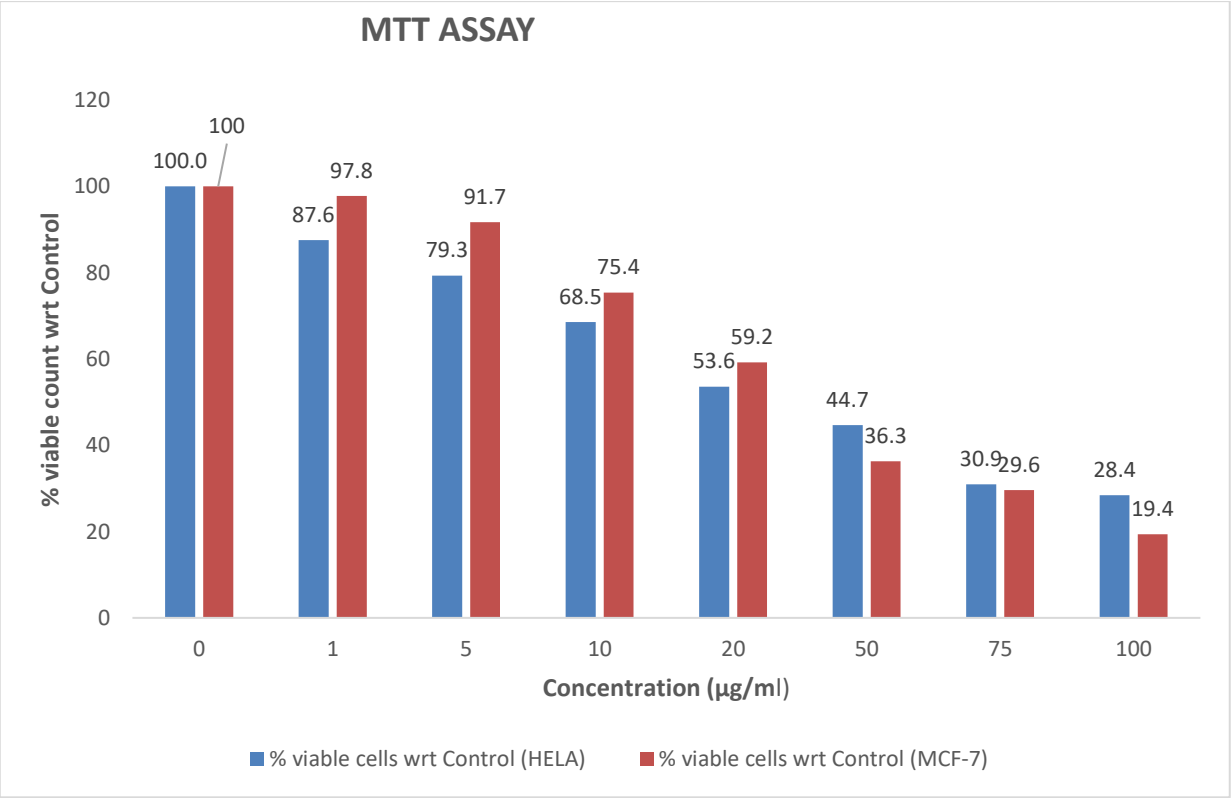

Figure 1. Dose-dependent in-vitro cytotoxic effect of *Acacia farnesiana* ethanolic bark extract on HeLa and MCF-7 cell lines (MTT assay)

CONCLUSION

The present investigation demonstrated that the ethanolic bark extract of *Acacia farnesiana* exhibits significant concentration-dependent cytotoxic activity against human breast (MCF-7) and cervical (HeLa) cancer cell lines. The extract effectively reduced cell viability in both cell lines, with a more pronounced inhibitory effect observed in MCF-7 cells, indicating greater susceptibility of breast cancer cells to the phytoconstituents present in the bark. The anticancer activity may be associated with the presence of phenolics, flavonoids, tannins, and other bioactive compounds capable of modulating oxidative stress, inducing apoptosis, and suppressing cellular proliferation. The results highlight the therapeutic potential of *Acacia farnesiana* as a natural source of anticancer agents. Although the study was limited to in vitro evaluation, the significant antiproliferative effects observed support further investigation into the molecular mechanisms underlying its activity, isolation of active constituents, and validation through in vivo and clinical studies. Overall, *Acacia farnesiana* bark represents a promising candidate for future development of plant-derived anticancer therapeutics.

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