



# ANTICANCER ACTIVITY, ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF LEUCAENA LEUCOCEPHALA'S ROOT EXTRACTION

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## ABSTRACT

Plants are a valuable resource for the identification of novel pharmaceutical products that can be used in the development of new drugs, and their secondary metabolites are special sources of medications, food additives, flavors, and other industrial uses. It is possible to predict a plant's pharmacological activity by identifying its phytochemicals. Plants are capable of creating secondary metabolites, according to phytochemicals. The protective and structural properties of phytochemicals contribute significantly to the lifetime of plants. A member of the Fabaceae family, *Leucaena leucocephala* is a medium-sized, quickly-growing tree. *Leucocephala*, which refers to the blooms, is the particular name for them. *Leu* is a Greek word that means white, and *cephala* means head. There have been allegations that the various sections of *L. leucocephala* possessed medicinal properties. Plant extracts have been demonstrated to possess strong antibacterial and antifungal activities. Alkaloids, flavonoids, saponins, tannins, and triterpenoids have all been cited as important phytochemical components of this plant. The pharmacological effects of this plant include antioxidant, anti-cancer, anti-inflammatory, anti-bacterial, and anti-diabetic characteristics. In this study, *Leucaena leucocephala* root extract was studied for antioxidant activity and anticancer activity. The *Leucaena leucocephala*'s root ethanolic extract showed the presence of tannins, terpenoids, steroids, glycosides, flavonoids, quinones, phenol, and carbohydrates. The antioxidant activity of *Leucaena leucocephala*'s root ethanolic extract was found to be 100.943 µg/ml. The anticancer activity of *Leucaena leucocephala*'s root extract was found to be 187.801 µg/ml. Thus, *Leucaena leucocephala* root extract will act as a good therapeutic agent with antioxidant and anticancer properties.

**KEYWORDS:** *Leucaena Leucocephala*, phytochemicals, antioxidant activity, anticancer activity.

## INTRODUCTION:

It is estimated that between 350,000 and roughly half a million species of vascular plants or 10% of all vascular plants are used as medicines. The use of plants as medicine dates back to prehistoric times and continues now [1]. Humans have always depended on nature to provide for their basic necessities, including food, clothes, housing, transportation, fertilizers, flavors, and medicines. Both conventional and Western

medicines heavily rely on plants [2]. In addition to offering food advantages and a variety of treatments, plants are vital to human existence. Due to their bioactive and medicinal qualities, various plant components, such as shrubs, herbs, and roots, have been used in our daily lives for many years [3]. Modern medical research focuses on using plants to extract essential components so that medications with precise metabolic intermediates can be created. It is estimated that around 50,000 plant species are used as medicines globally [4]. Any plant that has chemicals that can be utilized therapeutically or that contains building blocks for the creation of chemo- or pharmaceuticals is considered a medicinal plant. Around the world, people have traditionally utilized plants to cure a wide range of illnesses, including infectious diseases like diarrhea, fever, and colds as well as for birth control and dental hygiene. Additionally, a lot of the psychotropic compounds found in conventional medicine come from plants. A number of known therapeutic characteristics are produced by traditionally utilized medicinal herbs [5]. In many underdeveloped nations, herbal medicine continues to be the primary form of treatment. The prevalence of naturally occurring substances with medical characteristics has been connected to the widespread usage of herbal medicines and health care preparations, including those recorded in ancient scriptures like the Vedas, and are obtained from commonly used traditional herbs and medicinal plants. When natural products are combined to treat illnesses, some fascinating results have been observed, most notably the synergistic effects and poly-pharmacological use of plant extracts [6]. The use of various portions of various medicinal plants to treat various ailments has been popular since ancient times in India, where thousands of species of plants are known to have therapeutic value [7].

A variety of Indian medicinal plants have been utilized for centuries due to their helpful features to treat a variety of diseases [8]. Due to its anticancer, anti-diabetic, anti-inflammatory, antibacterial, antimicrobial, antifungal, antioxidant, and wound-healing capabilities, volatile oils, secondary metabolites, polypeptides, polysaccharides, and other natural plant products are employed [9]. Secondary metabolites found in plants, such as tannins, terpenes, polyphenols, glycosides, flavonoids, alkaloids, and a few more pigments, aid to guard against diseases and stressful conditions and support good health [10]. Any component of the flora can be used to produce plant metabolites [11]. The plant produced various chemical components known for their biological functions, including alkaloids, phenolic compounds, saponins, etc [12]. According to numerous research, plant extracts have antibacterial activity against both Gram-positive and Gram-negative bacteria. Many of these extracts show antibacterial activity that is comparable to or superior to that of common antibiotics [13]. These plant-based active ingredients benefit both human and other animal immunological, respiratory, excretory, neurological, and circulatory systems [14].

*Leucaena leucocephala* subsp. *glabrata*, sometimes known as giant leucaena, is a fast-growing, drought-tolerant, and disease-resistant tree legume that is commonly produced as wholesome farm animal fodder in tropical and subtropical regions. It can be repeatedly pruned to remain a bushy shrub with a lot of foliage, despite the fact that it eventually becomes a medium-sized tree. It is extremely resilient to a variety of environmental stresses, such as drought stress and soil pH that are both alkaline and acidic [15]. It can thrive in soil with degraded slopes and on mountainsides due to its extensive root system and capacity to fix nitrogen [16]. Mimosine, a nitrogen-rich nonprotein amino acid, is found in all parts of the plant and is produced in great quantities by the giant *Leucaena* [17]. In the villages of Tamil Nadu, India, *Leucaena leucocephala* (Lam.) de

Wit is a well-known fodder plant. However, this plant possesses medicinal qualities that are concealed within it. Plant metabolites are the cause of the medicinal effects. Native to Southern Mexico and Northern Central America, *Leucaena leucocephala* (Fabaceae) is a medium-sized, quickly-growing tropical tree [18]. It has now proliferated widely in many tropical and subtropical areas. The common names are White lead tree, White Popinac, Jumbay, and Wild Tamarind. In India, kubabul or subabul is a well-known name [19].

It was marketed as a "miracle tree" because of its amazing variety of uses [20]. The tree is useful for a variety of things, including preventing soil erosion and providing fuel, wood, fodder, and green manure [21]. The species, which is found in latitudes between 30 degrees north and south of the equator, is mostly a tropical species with weak cold resistance. It thrives in climates with an average annual temperature between 25°C and 30°C, between 650mm and 3000mm of precipitation, and up to 4-6 months of dry weather [22]. There are three recognized subspecies of *L. leucocephala*: ssp. *leucocephala*, which is shrubby and develops to a height of 5 meters, ssp. *glabrate*, which has a huge trunk and grows to a height of 20 meters, and ssp. *ixtahuacana*, which is medium-sized and grows to a height of 10 meters with a lot of branches [23]. The species has alternating, bipinnate leaves with 4–9 pairs of pinnae and 13–21 pairs of leaflets per pinnae, and alternate stems. In the twilight, the leaves exhibit nyctinasty [24].

Under stress situations like low temperatures and extreme aridity, it is an evergreen plant that can turn facultatively deciduous. Fast-growing species *L. leucocephala* can achieve reproductive maturity in 12 months, or 4 months in optimum conditions [25]. The young branches on flower heads actively develop to a diameter of 12 to 21 and yield 100 to 180 blooms each. 5–20 pods are produced by one flower head. Pods are 11–19 cm in length and 15–21 mm in width, and include 8–18 seeds each. The plant blooms continuously and produces a large number of seeds, primarily by self-fertilization [26].

The tropics and subtropics currently have a more evenly dispersed population of species. *L. leucocephala* has probably spread as a result of its favorable traits. The species is well-known for producing wholesome, superior-quality fodder trees throughout the tropics and subtropics. Both ruminant (such as cattle, water buffalo, and goats) and non-ruminant (such as rabbits, poultry, and fish) animals use it as a feed component [27]. The leaves of the species contain significant amounts of calcium and phosphorus as well as key amino acids including phenylalanine, leucine, isoleucine, and histidine. Protein accounts for 22–28% of the dry weight of the leaves [28]. Its medium-density, durable wood is suitable for use in carpentry and includes up to 20% of dry weight in carbs. It provides around 4600 calories per kilogram when used as fuel, and its biochar improves the soil condition of agricultural areas [29]. Plantations for cocoa, coffee, and tea all employ *Leucaena leucocephala* as a shade tree. Additionally, it provides diverse crops with a shelterbelt [30]. The plant may be used to restore vegetation to degraded areas, watersheds, and slopes in order to reduce erosion and promote the establishment of vegetation [31].

There are many local people from the eastern and northeastern states that utilize *L. leucocephala* for medicinal purposes, demonstrating the relevance of this plant from an ethnopharmacological perspective. *L. leucocephala* has traditionally been used as livestock feed. According to studies, *L. leucocephala* may have anti-diabetic and anti-emetic properties [32]. The usage of plant materials has expanded from the generation of methane to include high-quality animal feed. *L. leucocephala* seeds are rich in protein (between 24.5% and 46%), several

important amino acids, and beta-carotene [33]. Additionally, its leaves and young pods are used as vegetables by natives in Central America and South Asia. Its pods, leaves, and bark are used to create the colours brown, red, and black in Mexico. The species' bark and roots are used as a folk remedy as well, and the species' roots are said to cause abortions [34]. *L. leucocephala* seed gums are used to treat digestive system disorders and serve as laxatives, while the whole seed has been used as a replacement for coffee [35].

The polysaccharides in *L. leucocephala* seed gum are responsible for the plant's ability to prevent cancer and suppress cell proliferation [36]. The tree also possesses a wide range of biological properties, such as antiviral, antidiabetic, anti-inflammatory, anti-cancer, antithrombotic, anticoagulant, and immunostimulant features [37]. Our body generates highly reactive free radicals such as the hydroxy radical, superoxide anion, and singlet oxygen during normal metabolism. These free radicals are normally neutralized by endogenous antioxidants. When the generation of free radicals rises, antioxidants are reduced [38].

These free radicals then damage lipids, proteins, and DNA, which results in a number of diseases including cancer, diabetes, asthma, neurological disorders, and myocardial infarction. Antioxidants are thus required to either neutralize or scavenge these free radicals. Since the start of civilization, medicinal plants have been used to treat and prevent sickness. Numerous studies have validated their role as natural antioxidants [39]. Even in the current medical system, people still prefer to use plant-based remedies because they are safer, cheaper, and more readily available than manufactured drugs. Plants have a lot of secondary metabolites, such as phenol, flavonoids, alkaloids, glycosides, tannins, and others. As per studies, the phenol and flavonoids found in different plant parts have a defensive impact against diseases welcomed on by oxidative pressure [40].

Phytochemicals are compounds produced by a plant's normal metabolic activities. The complexity of phytochemicals varies depending on the kind of plant and the stage of growth. Some of the roles of these secondary metabolic products in plants include medicinal benefits [41]. The concept of developing synthetic and semi-synthetic versions of plant-based compounds for medicinal use first emerged in the 20th century. Researchers in the medical and food sectors have paid more attention to phytochemicals since these compounds offer the most potent therapeutic advantages [42]. In order to identify the sources of compounds that are relevant for both industrial and medicinal applications, screening of phytochemicals in dietary plants is essential. To find secondary metabolites in plants, a few crucial procedures must be carried out [43]. Plant species of all kinds contain many types of phytochemicals. Every single one of these is unique and fulfills a certain function. Among these functions are dietary benefits, physiological processes, phytotoxicity, antinutritional properties, pro-oxidants, anti-oxidants, anti-carcinogenic effects, analgesic effects, anti-inflammatory effects, and other therapeutic effects [44]. Cancer is a major public health concern and one of the top causes of mortality in the globe. Despite the fact that the disease is becoming more common, Central and South America, Africa, and Asia still account for almost 70% of all cancer-related deaths globally [45].

However, various phytochemicals and natural antioxidants have recently been recommended as anti-cancer adjuvant treatments due to their anti-proliferative and pro-apoptotic properties [46]. The continued search for anticancer medications or compounds from plants played a key part in finding alternative methods to have safe and lessen the side effects produced by chemotherapy since natural herbal therapies offer numerous advantages [47]. Over the past few decades, 200 unique chemical compounds have been licensed to treat cancer, 50% of which are generated from naturally occurring molecules that have had their structural makeup changed to make them useful and safe [48].

Due to their unique structural makeup, organic substances including terpenes, flavonoids, alkaloids, lignans, saponins, vitamins, glycosides, oils, and other secondary metabolites are essential in the selective repression of proliferation and activation of malignant cell death [49].



The leaves and seeds of *Leucaena leucocephala* are abundant in lipids, crude protein, tannin, carbohydrates, and mimosine. Mimosine, a non-protein amino acid, has the exact same chemical structure as tyrosine. Despite being deadly to animals, mimosine may be removed from leaves by soaking them in water for 24 hours [50]. However, mimosine has an anti-cancer impact by preventing the advancement of the cell cycle in human lung cancer cells [51]. *Leucaena leucocephala*'s medicinal uses include antimicrobial, anthelmintic, antibacterial, anti-proliferative, anti-diabetic, diuretic, anti-inflammatory, antioxidant, antitumor, antihistaminic, nematicide, pesticide, anti-androgenic, hypocholesterolemic, and hepatoprotective effects [52].

## MATERIALS AND METHODS:

### ROOT COLLECTION

*Leucaena leucocephala* root samples were collected from V.S.B Engineering College, Karur, Tamilnadu, India. Then the roots were dried under sunlight for about 2-3 days.



Fig 1: *Leucaena Leucocephala* tree



Fig 2: Root



**Fig 3: Dried root****Fig 4: Grained root****EXTRACTION OF ROOT**

The dried roots were coarsely powdered using an electric mixer. The sieved root mixture (1gm) was dissolved in ethanol(30ml) and the filtered root extraction was kept in a test tube at normal room temperature overnight. Then the extract was filled in Eppendorf tubes using a micropipette and it gets evaporated. After evaporation, ethanol was again added in Eppendorf tubes in the concentration of (10mg/ml) and agitated using a vortex machine. The extract was then stored and used.

**Fig 5: Extraction of root****METHODOLOGY****QUALITATIVE PHYTOCHEMICAL ANALYSIS:**

The extract solution was prepared by dissolving 200mg of the extract in 20ml of distilled water

Concentration: 10 mg /ml

The extracts were subjected to a qualitative phytochemical analysis by using below-standard tests.

➤ **Detection of saponins (foam test)**

2 ml of extract was added with 2 ml of distilled water and shaken vigorously; the formation of foam indicates the presence of saponins.

➤ **Test for Tannin**

1 ml of the extract was mixed with 2 ml of  $FeCl_3$ . The formation of greenish-black coloration indicates the presence of tannins.

➤ **Detection of Terpenoids & steroids -Salkowski test**

1 ml of the extract was mixed with chloroform and concentrated H<sub>2</sub>SO<sub>4</sub>. An appearance of the reddish-brown color of the interface indicates the presence of terpenoids. In the lower chloroform layer, red color appears that indicates the presence of steroids.

#### ➤ **Test for Glycosides**

2 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added to the extract. A reddish-brown color formed which indicated the presence of glycoside.

#### ➤ **Test of flavonoids – Alkaline reagent test**

2 ml of extract is added with sodium hydroxide, and dilute hydrochloric acid Formation, and the disappearance of the yellow color indicates the presence of flavonoids in the sample extract.

#### ➤ **Test for Alkaloids**

2 ml of extract was added with the concentrated hydrochloric acid. Then few drops of Mayer's reagent were added. The presence of green color or white precipitate indicates the presence of alkaloids.

#### ➤ **Test of Quinones**

1 ml of extract was mixed with concentrated sulphuric acid. The formation of red color indicates the presence of quinones.

#### ➤ **Test of Phenols-Ferric chloride test**

2 ml of distilled water followed by 3-4 drops or 2 ml of 10% ferric chloride were added to 1 ml of the extract. The formation of blue or green or bluish-black color indicates the presence of phenols.

#### ➤ **Test of Coumarins**

1 ml of sodium hydroxide was added to the extract. The formation of yellow color indicates the presence of Coumarins.

#### ➤ **Test of Carbohydrates**

1 ml of Molisch reagent was added to extract .1ml of concentrated H<sub>2</sub>SO<sub>4</sub> was carefully added. The mixture was then allowed to stand for 2 to 3 minutes. The formation of a purple or red or dull violet color at the interphase of the two layers indicates the presence of carbohydrates.

### **PHYTOCHEMICAL ANALYSIS:**

The extracts were subjected to a qualitative phytochemical analysis by using below-standard tests. The formation of greenish-black color indicates the presence of tannins. The presence of terpenoids is confirmed by the formation of reddish-brown color and the appearance of red color indicates the presence of steroids. The

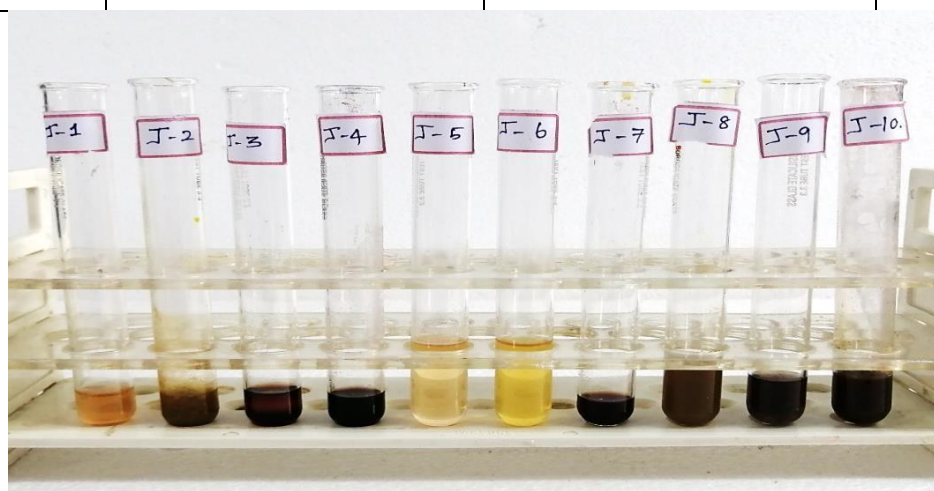
formation of reddish-brown color indicates the presence of glycoside. The formation and disappearance of yellow color indicates the presence of flavonoids. The presence of quinones is confirmed by the appearance of red color. The formation of blue or green or bluish-black colors confirms the presence of phenol. The appearance of yellow color indicates the presence of coumarins. A purple or red or dull violet indicates the presence of carbohydrates.

The formation of yellow colour indicates the presence of coumarins .The formation of foam indicates the presence of saponins.The formation of green or white precipitate confirms the presence of alkaloids.

In this study, the ethanolic root extract of *Leucaena leucocephala* revealed the presence of tannins, terpenoids, steroids, glycosides, flavonoids, quinones, phenol, and carbohydrates.

**Table 1: Phytochemical qualitative analysis**

S.NO	Test Name	Ethanolic Extract
1.	Saponins	Absent
2.	Tannin	Present
3.	Terpenoids	Present
4.	Steroids	Present
5.	Glycosides	Present
6.	Flavonoids	Present
7.	Alkaloids	Absent
8.	Quinones	Present
9.	Phenol	Present
10.	Coumarins	Absent
11.	CHO	Present



**Fig 6: Phytochemical analysis**



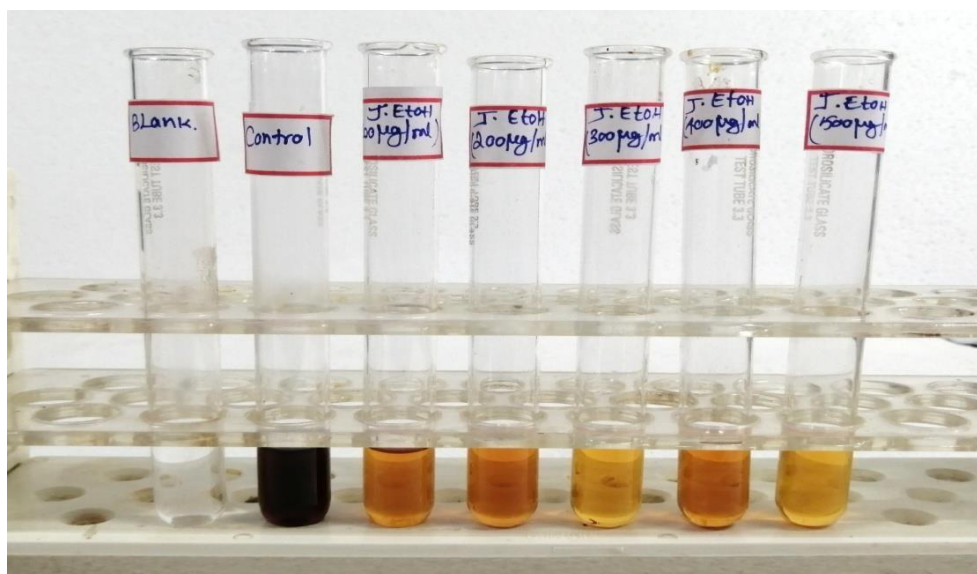
**ESTIMATION OF ANTI-OXIDANT ACTIVITY -DPPH radical****scavenging assay**

- About 100,200,300,400,500 µg/ml of extract solution aliquots were pipetted out into a series of test tubes.
- A stock solution of DPPH (1mM) was prepared in methanol.
- The volumes of all the tubes were made up to 1.0 ml with distilled water.
- To all the tubes 2 ml of DPPH was added to all tubes and kept undisturbed at room temperature in darkness for 10 minutes.
- The reagent blank was prepared without the test item
- Ascorbic acid was used as standard.
- Then samples were read at 520 nm.

**Table 2: Estimation of antioxidant-DPPH radical scavenging assay**

Concentration (µg/mL)	Absorbance		Average	Inhibition %
	I	II		
Control	0.573	0.572	0.573	0.00
100	0.291	0.286	0.289	49.607
200	0.262	0.257	0.260	54.672
300	0.183	0.178	0.181	68.472
400	0.174	0.169	0.172	70.044
500	0.135	0.129	0.132	76.943
Ascorbic acid	0.063	0.172	0.068	88.122

**Interpretation:** The IC<sub>50</sub> value for the given sample by performing DPPH radical scavenging assay was 100.943 µg/ml.



**Fig 7: Antioxidant activityANTI-CANCER ACTIVITY**

The evaluation of the anti-cancer activity of *Leucaena leucocephala* rootethanolic extract against MCF-7 cells by MTT assay

## CYTOTOXICITY ASSAY

### METHODOLOGY

The cytotoxicity effect of the sample was tested against MCF -7 cell line by MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Mossman,1983). The cells were seeded in 96-well microplates ( $1 \times 10^6$  cells/well) and incubated at  $37^\circ\text{C}$  for 48 h in 5%  $\text{CO}_2$  incubator and allowed to grow 70-80% confluence. Then the medium was replaced and the cells were treated with different concentrations of samples and incubated for 24 h. The morphological changes of untreated (control) and treated cells were observed under a digital inverted microscope (40X magnification) after 24 h and photographed. The cells were then washed with phosphate-buffer saline (PBS,  $\text{p}^{\text{H}} - 7.4$ ) and 20  $\mu\text{L}$  of (MTT) solution (5 mg/ml in PBS) was added to each well. The plates were then stood at  $37^\circ\text{C}$  in the dark for 2 h. The formazan crystals were dissolved in 100  $\mu\text{L}$  DMSO and the absorbance was read spectrophotometrically at 570 nm. The percentage of cell viability was calculated using the formula,

$$\text{Cell viability (\%)} = (\text{Absorbance of sample} / \text{Absorbance of control}) \times 100.$$

Concentrations ( $\mu\text{g/ml}$ )	Absorbance		Average	Cell viability(%)	Inhibition %
	I	II			
<b>Control</b>	0.745	0.761	0.753	100	0
<b>31.25</b>	0.685	0.697	0.691	91.766	8.234
<b>62.5</b>	0.58	0.561	0.571	75.830	24.170
<b>125</b>	0.31	0.301	0.306	40.637	59.363
<b>250</b>	0.183	0.196	0.190	25.232	74.768
<b>500</b>	0.097	0.082	0.090	11.886	88.114

**Table 3:** Results of cytotoxicity assay

**Interpretation:** The IC<sub>50</sub> Value of *Leucaena Leucocephala* Root Ethanolic Extract against MCF-7 was found to be 187.801  $\mu\text{g/ml}$ .

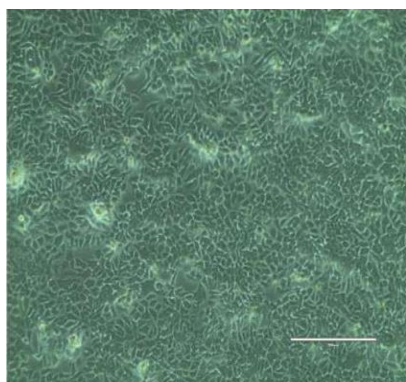


Fig 8: Morphology of control cells

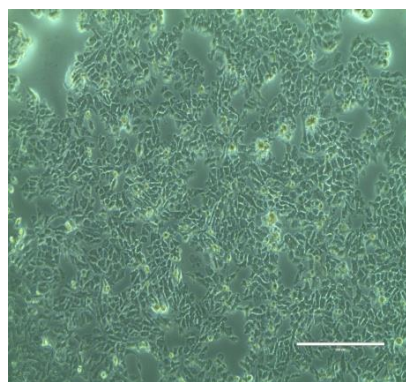


Fig 9: Morphology of cells treated with 31.25 $\mu\text{g/ml}$

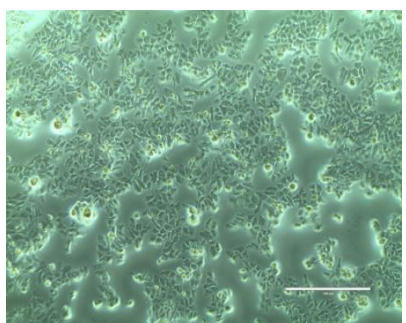


Fig 10: Morphology of cells treated with 125  $\mu\text{g/ml}$

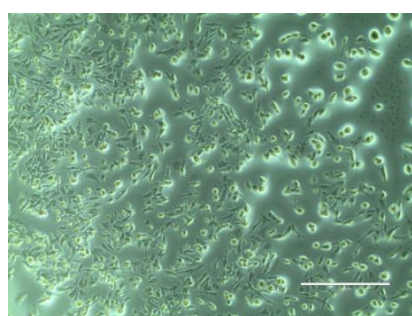


Fig 11: Morphology of cells treated With 62.50  $\mu\text{g/ml}$

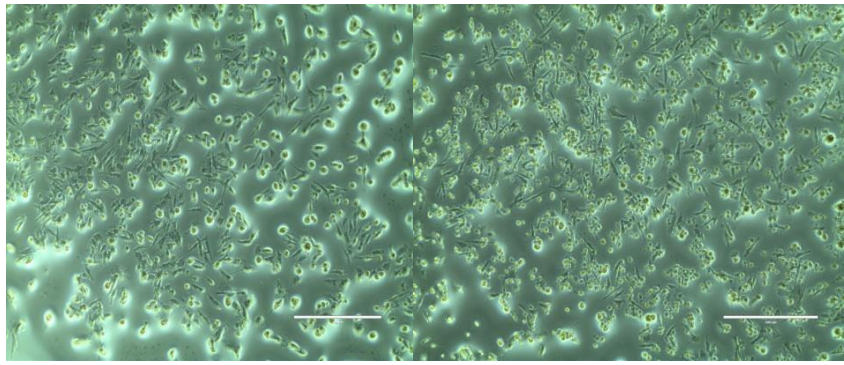


Fig 12: Morphology of cells treated  
With 250 µg/ml

Fig 13: Morphology of cells treated  
With 500 µg/ml

### Conclusion:

The root ethanolic extract of *Leucaena leucocephala* showed antioxidant and anticancer properties in the current investigation. *Leucaena leucocephala*'s root was ethanolicly extracted to reveal the presence of a number of phytochemicals, including tannins, terpenoids, steroids, glycosides, flavonoids, quinones, phenol, and carbohydrates. *Leucaena leucocephala* root ethanolic extract was reported to have 187.801 g/ml of anticancer activity against MCF-7 cells. By using a DPPH radical scavenging experiment, the root ethanolic extract of *Leucaena leucocephala* demonstrated 100.943 g/ml of antioxidant activity. As a result, the root extract of *Leucaena leucocephala* exhibits strong antioxidant and anticancer properties, making it a promising therapeutic agent for the treatment of numerous disorders.

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