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# ETHANOLIC EXTRACT OF PITHECELLOBIUM DULCE LEAVES AND STUDYING ITS IN-VITRO SKIN CANCER ACTIVITY

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## ABSTRACT

This study aimed to investigate the phytochemical composition, extraction, and in vitro cytotoxic and anti-skin cancer activity of *Pithecellobium dulce* leaf extract. Phytochemicals, renowned for their potential health benefits, were extracted from dried P. dulce leaves using ethanol via Soxhlet extraction, yielding 5.86% (w/w) of crude extract. Thin Layer Chromatography (TLC) revealed the presence of four distinct compounds with Rf values ranging from 0.664 to 0.849, suggesting a complex mixture. Gas Chromatography-Mass Spectrometry (GC-MS) further identified several compounds, including Benzene derivatives and Bromocyclopentane, indicating the chemical diversity of the extract. The in vitro cytotoxicity of the crude extract was evaluated using the MTT assay against both normal L929 fibroblast cells and A431 human skin cancer cells. Results showed that the extract exhibited relatively low toxicity towards normal L929 cells, with an IC50 value of 485.61~\mu g/mL. Importantly, the extract demonstrated significant anti-skin cancer activity against A431 cells, achieving an IC50 value of 169.58~\mu g/mL. These findings suggest that *Pithecellobium dulce* leaf extract possesses promising selective cytotoxic effects against skin cancer cells, highlighting its potential as a source of novel therapeutic agents for cancer treatment.

**KEYWORDS:** *Pithecellobium dulce*, Phytochemicals, Soxhlet extraction, Thin Layer Chromatography, Cytotoxicity, Anti-skin cancer, A431, L929.

## 1. INTRODUCTION

Skin cancer, particularly non-melanoma types such as basal cell carcinoma and squamous cell carcinoma, has seen a marked increase in incidence across the globe. While conventional chemotherapeutics and radiation therapies remain the mainstay of treatment, their adverse effects and rising resistance underscore the need for novel, natural anticancer agents with minimal side effects. Nature has long served as a rich reservoir of bioactive compounds, many of which have inspired modern drug development. Among them, *Pithecellobium dulce* (Roxb.) Benth, a leguminous plant commonly found in tropical and subtropical regions, has garnered attention due to its ethnomedicinal relevance. Traditionally used in folk remedies for treating wounds, inflammation, and gastrointestinal disorders, recent studies have suggested its leaves possess a myriad of pharmacological effects.

The leaves of P. dulce are abundant in polyphenols, flavonoids, and other antioxidant-rich phytochemicals, which are known to combat oxidative stress—a critical factor in cancer initiation and progression. However, its potential role in combating skin cancer, especially at the cellular level, remains underexplored.

This study aims to bridge that gap by investigating the ethanolic extract of P. dulce leaves for its in-vitro cytotoxic activity against skin cancer cells. The research includes extraction, phytochemical profiling, and cytotoxic evaluation using the MTT assay to determine the extract's anti-proliferative efficacy. The findings are expected to contribute to the growing body of evidence supporting the therapeutic use of plant-based compounds in oncological research.

## 2. MATERIALS AND METHODS

### 2.1 COLLECTION OF PLANT MATERIAL

Fresh leaves of *Pithecellobium dulce* were collected from local regions in \ [Chengalpattu district, Tamil Nadu state, India] were collected during 2025. The plant material was washed thoroughly, initially with tap water and then with distilled water to remove any debris or dust particles and was then allowed to dry in on oven at 40°C. the dried plant leaf material was ground to a fine powder and stored at room temperature in airtight containers until used further.

**Preparation of plant extract**

The extraction procedure for the isolation of phytocompounds from plant leaves has been practiced since long time. The precise mode of extraction depends on texture and water content of plant material being extracted and type of substance which is to be isolated. Normally crude extract is taken from Soxhlet apparatus using ethanol as a solvent. Ethanol shows good solubility for maximum organic compounds, and it can be easily recovere[1].

**Soxhlet extraction**

In this method, powdered leaves sample was placed in thimble chamber of the Soxhlet apparatus. The extraction solvent ethanol was heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. When the liquid content reached the siphon arm, the liquid contents emptied into the bottom flask again and the process was continued.

About 30 g of dried sample powder was weighed and extraction process is carried out by using 300 ml of ethanol in Soxhlet apparatus for 48 hours. The extract was concentrated by evaporation at 70 °C for 8 h and then dried. The concentrated extract was in dried form and stored at room temperature prior to phytochemical screening[2].

**2.2 PHYTOCHEMICAL ANALYSIS**

Table 1 shown Phytochemicals, the bioactive compounds found in plants, have garnered significant attention in both scientific research and the pharmaceutical industry due to their potential health benefits[5]. The analysis of these compounds is crucial for validating their therapeutic properties and exploring their applications in medicine[6].

**Importance of Phytochemical Analysis**

Phytochemical analysis is essential for understanding the chemical constituents of medicinal plants and their associated health benefits[4]. The importance of extracting, measuring, and identifying bioactive compounds, particularly phenolic compounds, which are prevalent in fruits and vegetables[7]. This analysis is critical for validating the health benefits of natural antioxidants and for promoting their use as alternatives to synthetic antioxidants like BHT and BHA[3]. An overview of phenolic compounds and flavonoids, emphasizing their health-promoting effects, including antioxidant, antibacterial, and anticancer properties. By illustrating examples from various medicinal plants, this research underscores the necessity of phytochemical analysis in the development of new medicinal applications[8].

**Table 1: Test Procedure for Phytochemicals Analysis.**

| S. No. | Phytochemicals | Procedure | Results |
|---|---|---|---|
| 1. | Alkaloids | 1 mL of extract was added with 2 drops of Mayer's reagent (added along sides of the test tubes) | Appearance of white creamy precipitate indicates the presence of alkaloids |
| 2. | Flavonoids | A few drops of dilute sodium hydroxide was added to 1 mL of extract | An intense yellow colour appeared in the plant crude extract, which became colourless on the addition of a few drops of dilute acid indicates the presence of flavonoids |
| 3. | Carbohydrates | 1 mL of extract was added with 1 mL of Benedict's reagent. The reaction mixture was boiled for 3 min in water bath. | Appearance of coloured precipitate indicates the presence of sugar |
| 4. | Proteins | 1 mL of extract was added with 500 µL of Ninhydrin reagent | Appearance of Purple colour indicates the presence of amino acids |
| 5. | Steroids | 1 mL of extract was added with 2 mL of chloroform. Then 2 to 3 mL of concentrated Sulfuric acid was added along side of the test tube. | Formation of bilayer (red top layer and greenish bottom layer) indicates the presence of steroids |
| 6. | Terpenoids | 1 mL of extract was mixed with 3 mL of chloroform and 2 mL of concentrated sulfuric acid along the side of the test tube, forming a layer. | A reddish-brown coloration at the interface between the chloroform and sulfuric acid layers indicates the presence of terpenoids |
| 7. | Tannins | A few drops of a 10% ferric chloride solution were added to 1 mL of extract | Development of a dark blue or green-black color indicates the presence of Tannins |
| 8. | Saponins | 1 mL of extract was added with 10 mL of distilled water and shaken in a graduated cylinder for 15 min. | A 2 cm layers of foam indicates the presence of saponins |
| 9. | Glycosides | 1 mL of extract was added with 3 mL of chloroform and mixed the contents for 3 min. after mixing, chloroform layer was separated and 10% ammonia solution was added. | Appearance of pink colour indicates the presence of glycosides |
| 10. | Phenols | A few drops of a 1% ferric chloride solution were added to 1 mL of extract | The formation of a red, blue, green, or purple coloration indicates the presence of phenols |

## 2.3 THIN LAYER CHROMATOGRAPHY ANALYSIS

The analytical TLC technique was used to count the number of compounds in the test sample. With the aid of capillary tubes, the test sample were spotted on the 0.25 mm pre-coated silica gel plates with a fluorescent indicator (F254) about 1 cm from the bottom edge of the plates and left to dry. The solvent systems (Mobile phase) of hexane/ethyl acetate in the ratio of 9: 1 was used[9]. A 356 nm UV lamp was used to visualize the dried plate. After that, the spots that had separated were marked, and the fronts of their samples and solvents were measured.

The following formula was used to determine the eluted spots' retention factor (Rf)[10]:

Rf = Distance traveled by solute/ Distance traveled by solvent front

## 2.4 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

Begin by stating that the "Extract" sample (Sample ID: 40674) was analyzed using a Gas Chromatography-Mass Spectrometry (GC-MS) system, specifically mentioning the GC-2010 Plus and GCMS-QP2010 Plus. Detail the injection parameters, including a 1.00 µL injection volume in split mode at 250.00 °C with a split ratio of 20.0. Describe the GC oven temperature program, noting the initial temperature of 50.0 °C held for 1.00 minute, followed by a ramp at 10.00 °C/min to 280.0 °C, held for 2.00 minutes. Mention the column flow of 1.00 mL/min with a linear velocity of 36.3 cm/sec, using helium as the carrier gas. For the MS conditions, specify the ion source and interface temperatures at 200.00 °C and 280.00 °C, respectively, and state that data was acquired in scan mode from m/z 40.00 to 900.00, starting at 3.50 minutes with a 3.00-minute solvent cut. Finally, include that compound identification was achieved by comparing the obtained mass spectra with a reference library, such as WILEY8.LIB

## 2.5 IN-VITRO CYTOTOXICITY ASSAY

### Cell Line Used

The non-cancerous L929 cell lines were sourced from the National Centre for Cell Science (NCCS), located in Pune, India[11].

### Cell Culturing and MTT Assay

Trypsinized cells were used in the monolayer cell culture. They were subcultured in Dulbeccos modified Eagles medium enriched with 10% FBS and 100 µL of the suspension contained about 10000 cells[12]. These cells were then seeded into a 96-well microtiter plate and incubated for 24 hours at 37°C with 5% CO2. When a partial monolayer had formed after 24 hours of incubation the culture was cautiously aspirated. Next 100 µL of the crude

metabolites at different test concentrations (500, 250, 125, and 62.25 µg/mL) were added to the cell suspensions partial monolayer for each cell line separately. For an extra twenty-four hours this mixture was incubated at 37°C in an atmosphere containing 5% CO2. Following the aspiration of the media containing crude metabolites 10 µL of MTT reagent (5 mg/mL) was added to each well. The wells were then incubated for three hours at 37 °C with 5% CO2. After incubation 100 µL of dimethylsulfoxide (DMSO) was added to dissolve the formazan. A microtiter plate reader was used to read the micro-titre plate at 570 nm in duplicates (n = 2) in order to calculate the inhibition percentage (Alley et al. (1986)[13].

**Percentage of Cytotoxicity = ($A_{Control} - A_{Test}$) / $A_{Control}$ × 100**

Where, $A_{Control}$ is Absorbance of Control, and $A_{Test}$ is Absorbance of crude metabolites.[14]

## 2.6 IN VITRO ANTI-SKIN CANCER ACTIVITY ASSAY (A431 CELL LINE)

The anti-skin cancer activity of the *Pithecellobium dulce* crude extract was evaluated against human skin cancer A431 cell lines, also procured from NCCS, Pune, India, using the MTT assay[15]. We used the MTT assay—a colorimetric test based on the compound 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide—to measure how our test compound affected the growth of cancer cells. First, we plated our cancer cell lines in 96-well plates, adding 100 µL of DMEM medium (with 10% fetal bovine serum) to each well[22]. After letting the cells settle overnight at 37 °C, we treated them for 24 hours with various doses of our test sample (ranging from 62.5 µg to 1000 µg/mL). As controls, we used doxorubicin at the same concentrations as a positive control, plain[16.]

DMEM as a negative control, and wells without any cells as blanks[17].

After treatment, we incubated the plates for another 48 hours in a humidified 5% $CO_2$ atmosphere at 37 °C. Then we added 20 µL of a 5 mg/mL MTT solution (in phosphatebuffered saline) to each well and let it sit for 4 hours. This step allows living cells to convert MTT into purple formazan crystals[18]. To dissolve those crystals, we added 150µL of DMSO to each well, covered the plates with foil, and let them sit in the incubator for 12 hours. Finally, we measured the amount of dissolved formazan by reading the absorbance at 590 nm on a microplate reader[19].

The cell growth inhibition was measured using below formula[20];

**Percentage of growth inhibition** = (Control O.D Value − Test Sample O.D Value) ÷

Control O.D Value × 100

**Statistical Analysis and IC50 Calculation**

To evaluate the cytotoxicity, morphological changes were analysed and the half maximum inhibitory concentration (IC50) value—a measure of the concentration that inhibits half of viable cell development—was found[21]. The mean standard error of the duplicate values was provided. The linear regression analysis was performed after the test sample were evaluated at various concentrations to obtain the half-maximal (IC50) inhibitory concentration value[23].

## 3. RESULT AND DISCUSSION

### 3.1 EXTRACTION PROCESS RESULT

The Soxhlet extraction of the powdered *Pithecellobium dulce* using ethanol yielded a crude extract with notable efficiency. A total of 1.76 grams of dried extract was obtained from 30 grams of plant material, corresponding to a percentage yield of 5.86% (w/w). During the extraction, the solvent in the siphon tube gradually became lighter in color, indicating the progressive depletion of soluble compounds from the plant matrix. The extraction was carried out over a period of 48 hours, with continuous recycling of the solvent ensuring maximal solubilization of the phytocompounds. After concentration of the solvent using a rotary evaporator, the crude extract appeared as a dried mass. Fig 1 The extract was stored in an airtight container and kept at 4°C for further phytochemical screening and biological evaluation.

The yield was calculated using the following formula:

$$\text{Percentage yield} = \left( \frac{\text{Weight of extract}}{\text{Weight of dry plant material}} \right) \times 100$$

**Figure No. 1: Crude extract of *Pithecellobium dulce* leaves.**

### 3.2 PHYTOCHEMICAL ANALYSIS REPORT

Fig 2 Phytochemical analysis plays a pivotal role in validating the health benefits of natural products and advancing drug discovery efforts. As research continues to evolve, it is crucial to address existing knowledge gaps and explore innovative methodologies to optimize phytochemical extraction and analysis. By fostering a multidisciplinary approach that bridges traditional knowledge with modern science, the field of phytochemistry can contribute significantly to enhancing human health and ecological sustainability. Table 2 shown the report of phytochemicals

**Table 2: Phytochemicals test report.**

| S. No. | Phytochemicals | Results |
|---|---|---|
| 1. | Alkaloids | + |
| 2. | Flavonoids | + |
| 3. | Carbohydrates | - |
| 4. | Proteins | + |
| 5. | Steroids | + |
| 6. | Terpenoids | + |
| 7. | Tannins | - |
| 8. | Saponins | - |
| 9. | Glycosides | - |
| 10. | Phenols | - |

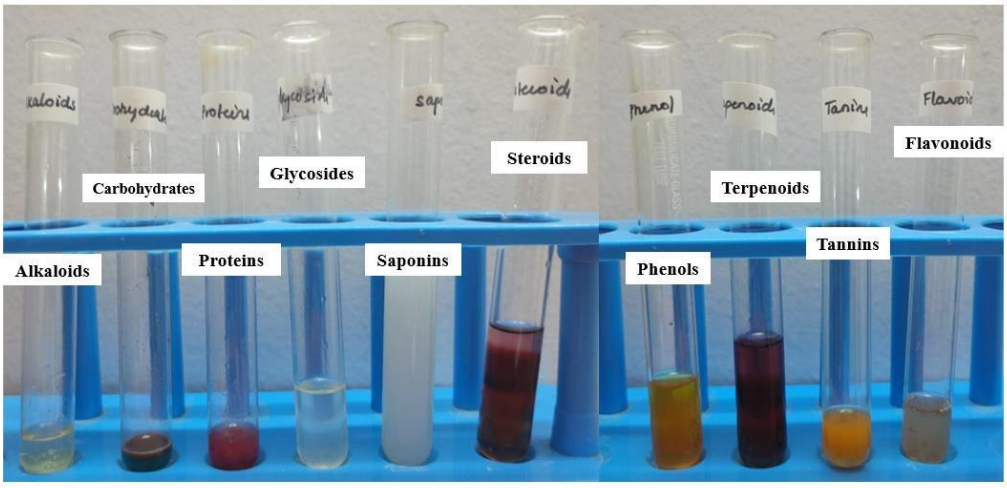



**Figure 2: Phytochemical test results.**

### 3.3 THIN LAYER CHROMATOGRAPHY (TLC) REPORT

The analytical TLC method was used to count the number of compounds in the test sample. The chromatographic spots which were illustrative of mixtures in the different concentrates

were noticed, and their Rf was calculated. The results of the TLC analysis are presented in Table 3. Four distinct spots with Rf values such as 0.664, 0.667, 0.788, and 0.849 cm were observed in the crude extract was shown in Figure 3. To completely separate the components, two-dimensional TLC, column chromatography, or high-pressure liquid chromatography (HPLC) may be required.

**Table 3: Compounds in crude extract retention factor (Rf) determined using TLC Plate.**

| Bands | Distance Travelled by band | Retention factor (in cm) |
|---|---|---|
| Band 1 | 2.324 cm | 0.664 |
| Band 2 | 2.335 cm | 0.667 |
| Band 3 | 2.761 cm | 0.788 |
| Band 4 | 2.973 cm | 0.849 |

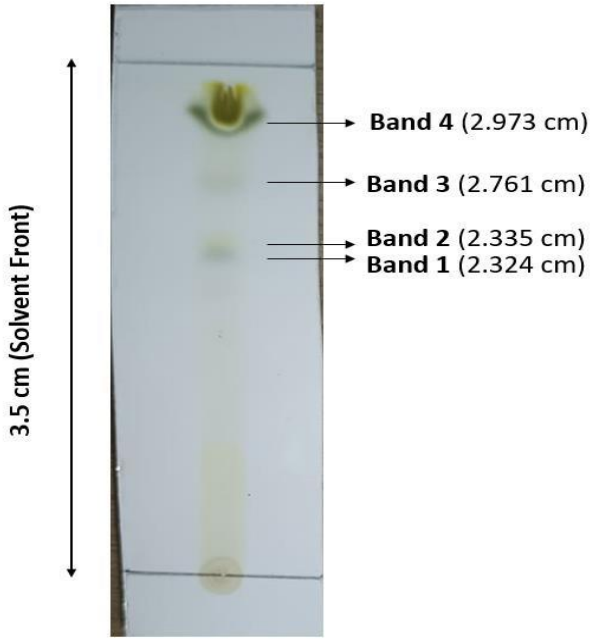



**Figure No.3: Thin Layer Chromatogram of crude extract.**

### 3.4. GC-MS ANALYSIS

GC-MS analysis provided detailed insights into the chemical constituents of the Pithecellobium dulce crude extract. Several compounds were identified based on their retention times and mass spectral data, matching with library databases. Notable identified compounds include: Benzene, 1,4-dimethyl- (Retention Time: 3.250 min), Benzene, 1-ethyl-2-methyl- (Retention Time: 6.273 min), and Bromo cyclopentane (Retention Time: 25.770 min). The Fig 4,5 showed various peaks, indicating the presence of a diverse range of compounds, each with unique retention times and mass fragmentation patterns. Table 4 shown a peak report of TIC.

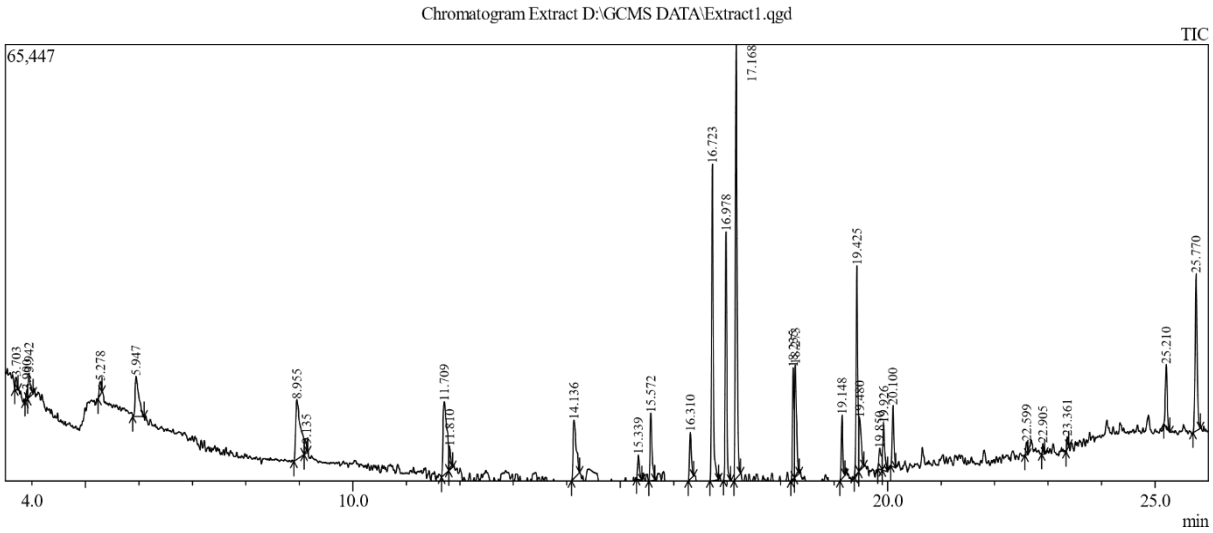



**Figure 4: Chromatogram Extract.**

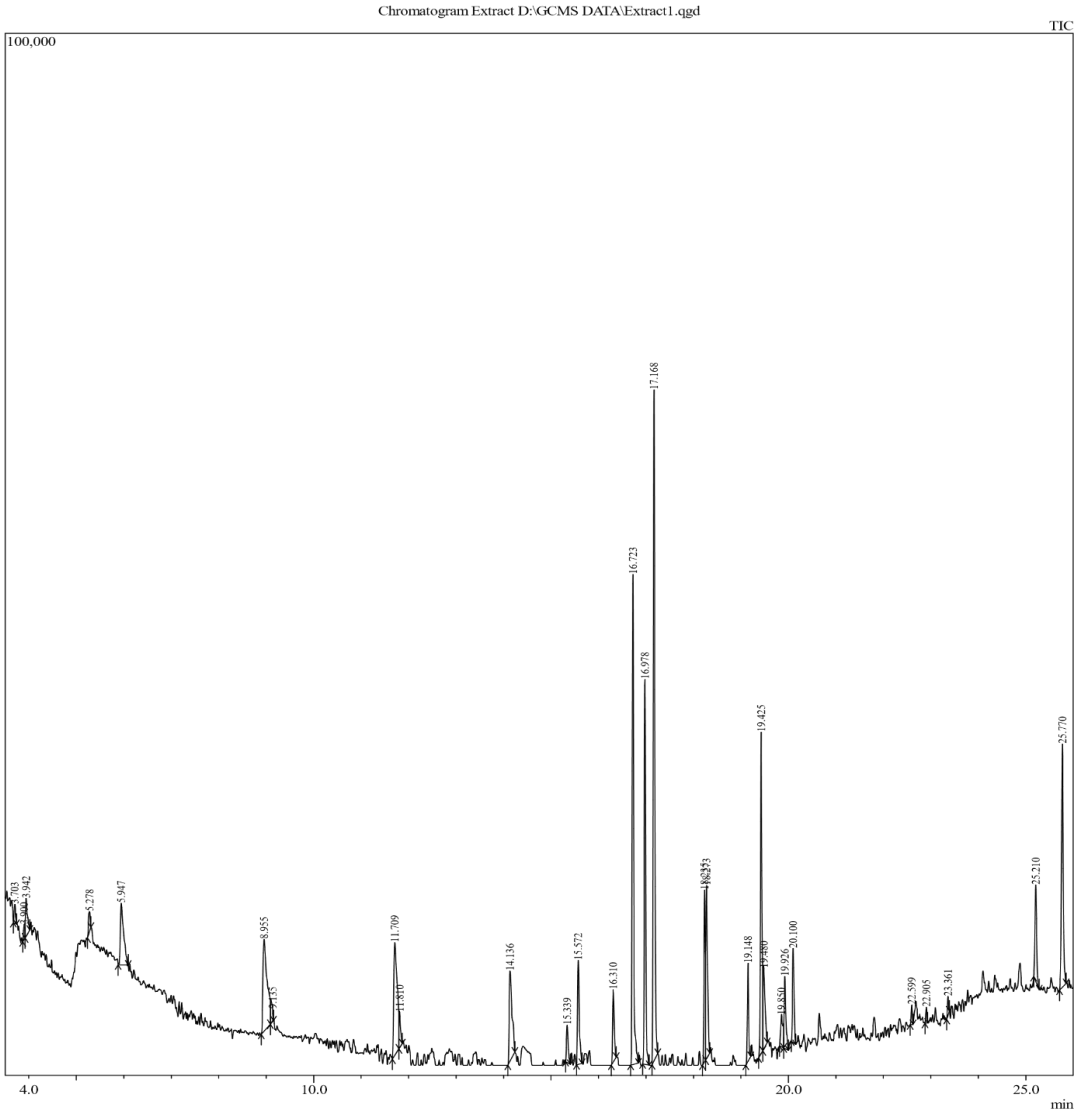



**Figure 5: Chromatogram Extract.**

**Table 4: Peak Report TIC.**

| Peak # | R. Time | Area | Area % | Height | Name |
|---|---|---|---|---|---|
| 1 | 3.703 | 3702 | 0.41 | 1828 | Acetamide, 2-cyano- |
| 2 | 3.900 | 3164 | 0.35 | 1436 | Acetamide, 2-cyano- |
| 3 | 3.942 | 10562 | 1.16 | 3650 | HYDROXYLAMINE, O-METHYL- |
| 4 | 5.278 | 5039 | 0.55 | 1964 | ETHANE, 1,1',1''-[METHYLIDYNETRIS(OXY)]TRIS- |
| 5 | 5.947 | 28690 | 3.16 | 5984 | Oxalic acid, butyl propyl ester |
| 6 | 8.955 | 55056 | 6.06 | 8922 | ISOBUTYRIC ACID, ALLYL ESTER |
| 7 | 9.135 | 5485 | 0.60 | 1232 | 4-T-BUTYL-1-TRIFLUOROMETHYLCYCLOHEXANOL |
| 8 | 11.709 | 55312 | 6.08 | 10924 | BUTANE, 2,2-DIMETHYL- |
| 9 | 11.810 | 7444 | 0.82 | 3431 | Propanoic acid, 2-hydroxy-, 2-methylpropyl ester |
| 10 | 14.136 | 35553 | 3.91 | 8738 | 1-METHYLBUTYL NITRITE |
| 11 | 15.339 | 8941 | 0.98 | 3621 | ISOXAZOLE, 3-(3-BUTENYL)-5-METHYL- |
| 12 | 15.572 | 25732 | 2.83 | 10062 | 2-NONENAL, 2-PENTYL- |
| 13 | 16.310 | 17117 | 1.88 | 7017 | 1-METHYLBUTYL NITRITE |
| 14 | 16.723 | 103652 | 11.40 | 47493 | 6-OCTEN-1-OL, 3,7-DIMETHYL-, ACETATE |
| 15 | 16.978 | 76285 | 8.39 | 37311 | 6-OCTEN-1-OL, 3,7-DIMETHYL-, ACETATE |
| 16 | 17.168 | 144964 | 15.95 | 65019 | 6-OCTEN-1-OL, 3,7-DIMETHYL-, ACETATE |
| 17 | 18.235 | 32526 | 3.58 | 16619 | 2H-PYRAN-2-ONE, TETRAHYDRO-6-(2-PENTYNYL)- |
| 18 | 18.273 | 39827 | 4.38 | 16772 | HEPTADECANOIC ACID, ETHYL ESTER |
| 19 | 19.148 | 18592 | 2.05 | 9492 | 6-(AZIDOMETHYL) TETRAHYDROPYRAN-2-ONE |
| 20 | 19.425 | 66784 | 7.35 | 31169 | HYDROGENATED NEROLOXIDE |
| 21 | 19.480 | 23685 | 2.61 | 7819 | BUTANE, 1-BROMO-2-METHYL- |
| 22 | 19.850 | 10497 | 1.15 | 3059 | 1-BROMO-2,2,3-TRIMETHYLCYCLOPROPANE |
| 23 | 19.926 | 17298 | 1.90 | 6994 | (4Z,9Z)-TETRADECA-4,9-DIEN-1-OL |
| 24 | 20.100 | 20241 | 2.23 | 9105 | HEPTADECANOIC ACID, ETHYL ESTER |
| 25 | 22.599 | 3421 | 0.38 | 1921 | HEPTANAL, 4-METHYL-4-NITRO-5-OXO- |
| 26 | 22.905 | 2236 | 0.25 | 1550 | 1,1-DIISOBUTOXY-BUTANE |
| 27 | 23.361 | 3082 | 0.34 | 1824 | CYCLOPROPANE, [[(1,1-DIMETHYLETHYL) SULFINYL]METHYL]- |
| 28 | 25.210 | 22599 | 2.49 | 9368 | 4,8-DIMETHYL-3(E),7-NONADIENYL THIOACETATE |
| 29 | 25.770 | 61553 | 6.77 | 23589 | DOCOSANE |
| | | 909039 | 100.00 | 357913 | |

### 3.5 IN-VITRO CYTOTOXICITY ASSAY

The MTT assay calculates the rate of cell division on the other hand a decrease in metabolic activity eventually results in necrosis or apoptosis which indicates a decline in cell viability. The MTT assay gauges the rate of cell division and when metabolic activity declines it ultimately results in necrosis or apoptosis which indicates a decline in cell viability. Even at higher concentrations (1000 µg, 500 µg and 250 µg) the crude metabolites as determined by the MTT assay showed less growth inhibition and no discernible cytotoxic effect on the L929 cell line. When it came to L929 cell lines the cytotoxic effects of the crude metabolites differed from those of regular doxorubicin

**Table 5: Percentage of Cytotoxicity by Crude extract.**

| Concentration | T1 | T2 | Average | % of Cytotoxicity |
|---|---|---|---|---|
| **62.5 µg/mL** | 0.376 | 0.351 | 0.3635 | 21.23510293 |
| **125 µg/mL** | 0.267 | 0.259 | 0.263 | 43.01191766 |
| **250 µg/mL** | 0.199 | 0.197 | 0.198 | 57.0964247 |
| **500 µg/mL** | 0.172 | 0.155 | 0.1635 | 64.57204767 |

**Table 6: Percentage of Cytotoxicity by Doxorubicin.**

| Concentration | T1 | T2 | Average | % of Cytotoxicity |
|---|---|---|---|---|
| **62.5 µg/mL** | 0.432 | 0.423 | 0.4275 | 7.86637931 |
| **125 µg/mL** | 0.383 | 0.382 | 0.3825 | 17.56465517 |
| **250 µg/mL** | 0.276 | 0.287 | 0.2815 | 39.33189655 |
| **500 µg/mL** | 0.229 | 0.223 | 0.226 | 51.29310345 |

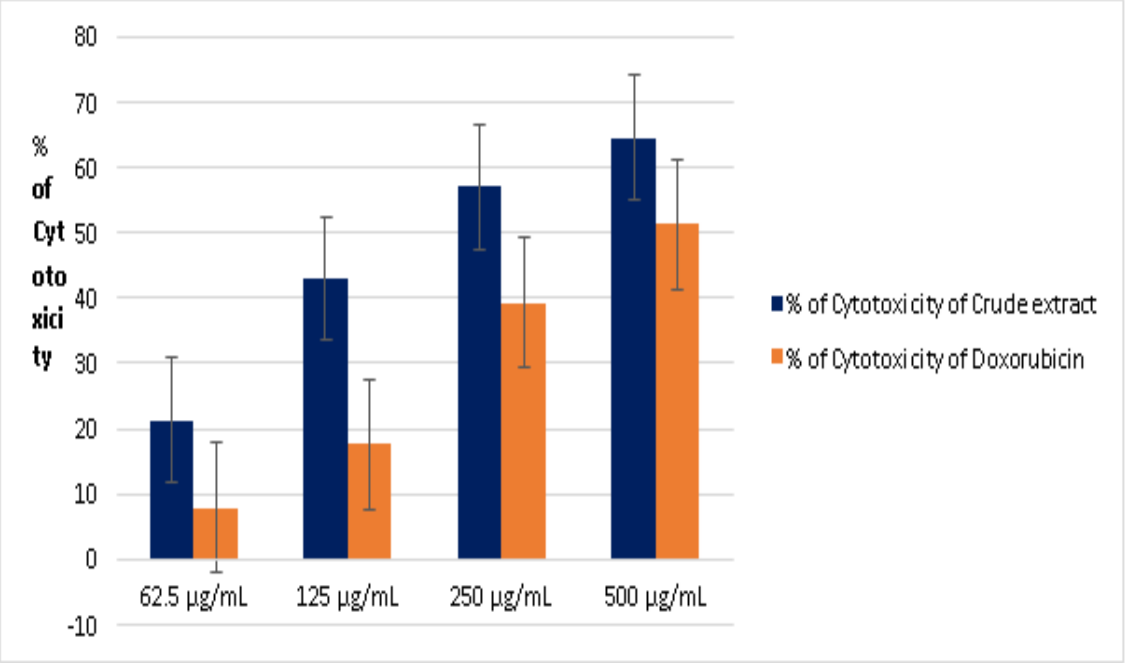
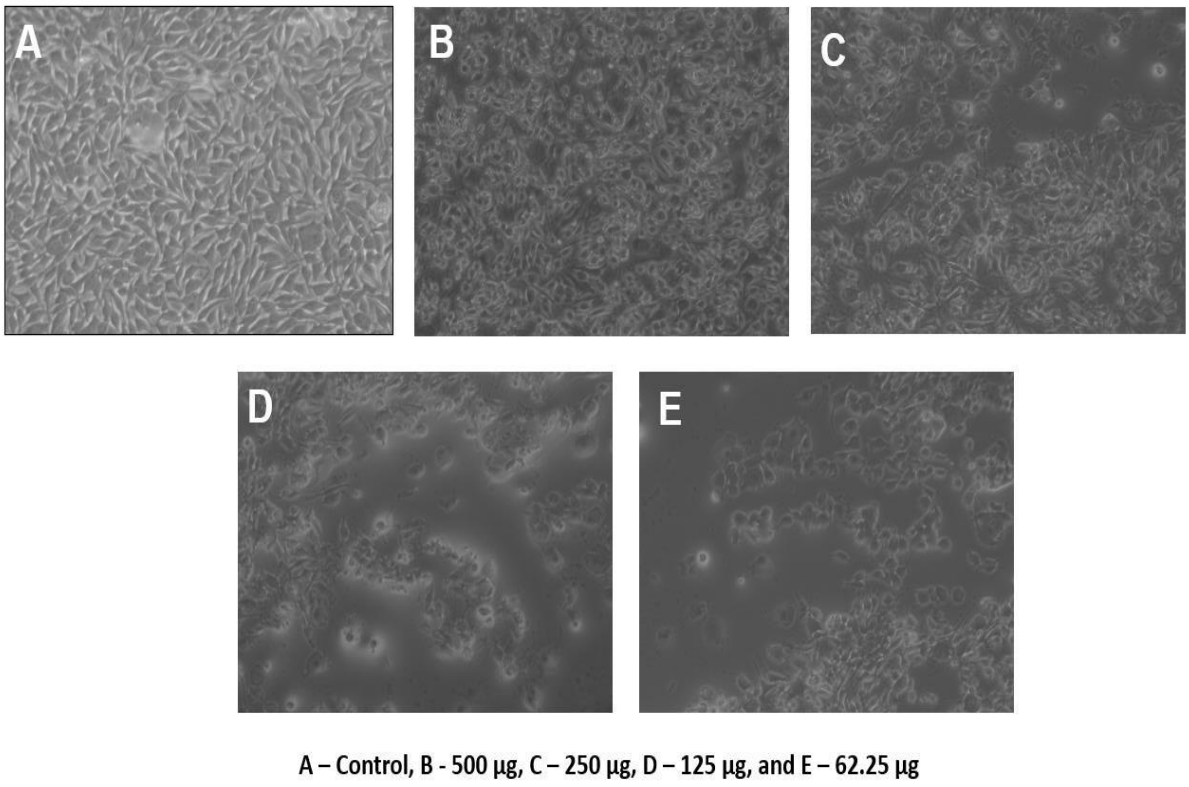



**Figure 6: Cytotoxicity effect of Crude extract in comparison with Doxorubicin IC50.**

The Fig 6 showed negligible toxicity against normal cell line L929, where in the tested concentration (62.5 µg/mL) showed 21.24% cytotoxicity with an IC50 value of 485.61 µg




A – Control, B - 500 µg, C – 250 µg, D – 125 µg, and E – 62.25 µg

**Figure 7: In vitro morphological profile of L929 cell observed under an inverted microscope at various concentrations compared to the control (without the crude extract and with the standard drug doxorubicin), after 24 hours of treatment. Representative photographs of morphological changes induced by crude extract after 24 h (indicate normal cellular morphology; altered cellular morphology with apoptosis; and altered cellular morphology.**

### 3.6 ANTI-SKIN CANCER ASSAY REPORT

We tested the cytotoxic effects of our sample on A431 human skin cancer cells using a range of concentrations. Interestingly, the test sample showed significant anti-cancer activity than the standard drug, doxorubicin. The concentration at which the sample reduced cell viability by 50% ($IC_{50}$) was calculated to be 137.53 µg/mL. At this dose, we observed a significant drop in cell viability compared to the untreated control group.

These results suggest that the test sample could have potential as a therapeutic option for treating skin cancer (see Figure 8).

**Table 7: O.D. Values of test sample treated A431 Cells.**

| Concentration of test sample (µg/mL) | O.D Values at 595 nm | | | | |
|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Average OD | Standard Error | Anti-cancer activity (%) |
| 1000 | 0.125 | 0.129 | 0.127 | 0.002 | 78.12230835 |
| 500 | 0.189 | 0.185 | 0.187 | 0.002 | 67.78639104 |
| 250 | 0.243 | 0.249 | 0.246 | 0.003 | 57.62273902 |
| 125 | 0.379 | 0.382 | 0.3805 | 0.0015 | 34.45305771 |
| 62.5 | 0.491 | 0.476 | 0.4835 | 0.0075 | 16.70973299 |

**Table 8: O.D. Values of standard drug (Doxorubicin) treated A431 Cells.**

| Concentration of Doxorubicin (µg/mL) | O.D Values at 595 nm | | | | |
|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Average OD | Standard Error | Anti-cancer activity (%) |
| 1000 | 0.114 | 0.119 | 0.086 | 0.0025 | 81.7989418 |
| 500 | 0.136 | 0.149 | 0.1425 | 0.0065 | 69.84126984 |
| 250 | 0.152 | 0.161 | 0.1565 | 0.0045 | 66.87830688 |
| 125 | 0.238 | 0.246 | 0.242 | 0.004 | 48.78306878 |
| 62.5 | 0.326 | 0.347 | 0.3365 | 0.0105 | 28.78306878 |

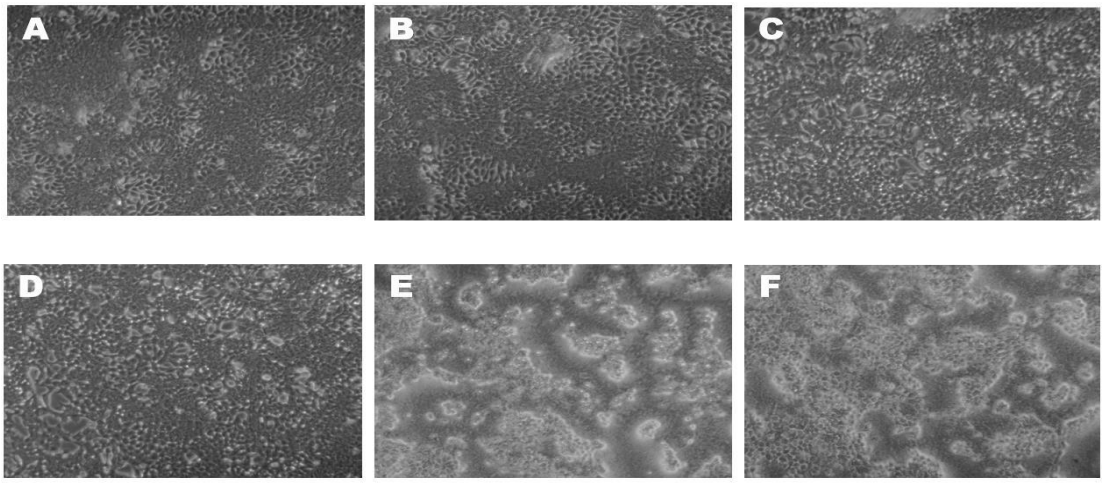



**Figure No.8: MTT assay measurement of cell survival. MTT Assay demonstrating the impact of various test sample concentrations on A431 cell lines over a 24-hour period. MTT is 3-(2,5-diphenyltetrazolium bromide)-3-(4,5-dimethylthiazol-2-yl). (A) Control cells, (B) 62.5 µg/mL treated cells, (C) 125 µg/mL treated cells, (D) 250 µg/mL treated cells, (E) 500 µg/mL treated cells, and (F) 1000 µg/mL treated cells. At higher concentrations of the test sample (500 and 1000 µg/mL), there was a noticeable changes cell size and structure. Around 50–60% of the cells exhibited membrane blebbing—small protrusions on the cell membrane—and ballooning was also evident. Furthermore, signs of apoptosis were observed in the cells treated with the test sample.**

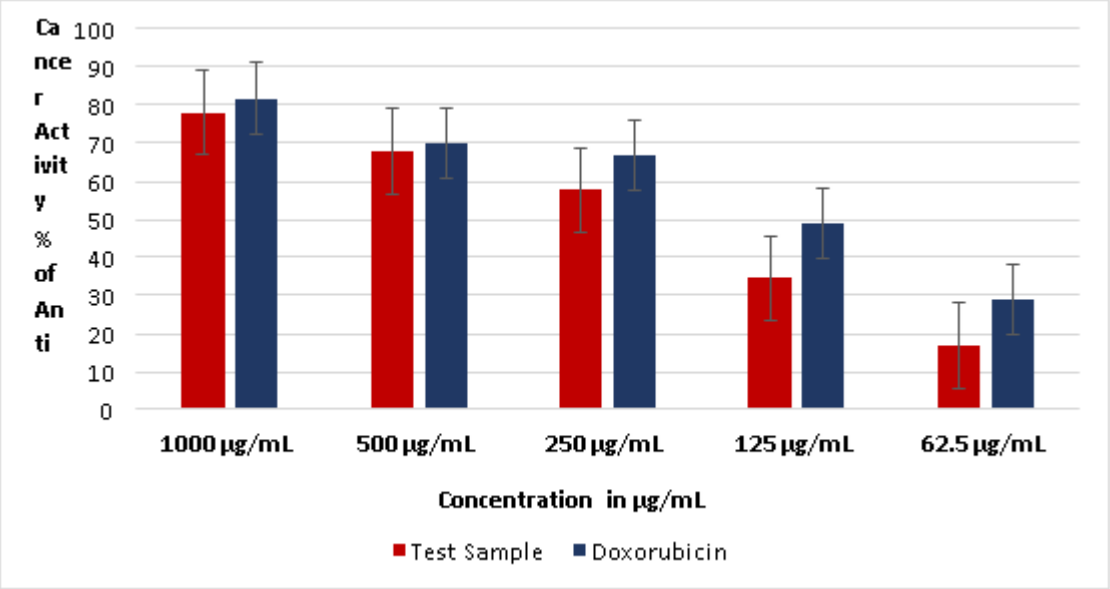



**Figure No. 9: Percentage of Cytotoxicity Effect of test sample in A431 cell line. The cytotoxicity effect was compared with standard drug (Doxorubicin).**

## CONCLUSION

This study successfully demonstrated the ethanolic extraction and preliminary phytochemical characterization of Pithecellobium dulce leaf extract. TLC and GC-MS analyses confirmed the presence of a diverse array of phytocompounds within the crude extract. Crucially, the in vitro assays revealed that the P. dulce extract possesses promising anti-skin cancer activity against A431 human skin cancer cells, with a favorable IC50 value, while exhibiting relatively low cytotoxicity towards normal L929 cells. These findings underscore the potential of Pithecellobium dulce as a valuable source of natural compounds with selective anticancer properties. Future research should focus on the isolation and structural elucidation of the specific active compounds responsible for the observed anticancer activity, followed by detailed in vitro mechanistic studies to understand their mode of action. Furthermore, in vivo studies are essential to validate the efficacy and safety of the extract and its isolated compounds in a more complex biological system, paving the way for the development of novel plant-derived therapeutic agents for skin cancer.

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## CONFLICTS OF INTEREST

The author(s) declares no conflicts related to the formulation testing or reporting of this project.

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