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INVITRO LIVER CANCER POTENTIAL OF ETHANOLIC EXTRACT OF TRIBULUS LANUGINOSUS

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ABSTRACT
The current investigation was conducted to assess the ethanolic extract of *Tribulus lanuginosus* in vitro anticancer potential against liver cancer cell lines. A significant worldwide health issue linked to oxidative stress, persistent inflammation, and increasing liver damage is liver cancer, especially hepatocellular carcinoma. The hepatoprotective and anticancer potential of medicinal plants that include bioactive phytochemicals including flavonoids, saponins, and phenolic compounds is being studied more and more. *Tribulus lanuginosus* leaves were gathered, verified, shade-dried, and extracted with ethanol in this study. Initial phytochemical screening verified the existence of significant secondary metabolites. After that, the extract's cytotoxic activity was assessed on liver cancer cell lines using conventional in-vitro techniques. Significant anticancer potential was shown by the results, which showed a dose-dependent inhibitory effect on cancer cell survival that may be connected to cytoprotective and antioxidant pathways. Overall, the study supports *Tribulus lanuginosus* potential use as a natural source of hepatoprotective and anticancer drugs; nevertheless, more research is advised to identify active chemicals and verify efficacy through sophisticated experimental and clinical studies.

KEYWORDS: *Tribulus lanuginosus*; Liver cancer; Hepatocellular carcinoma; In-vitro anticancer activity, Ethanolic extract; Phytochemical screening; Cytotoxicity; Antioxidant activity.

INTRODUCTION

- Liver cancer is one of the most common and life-threatening malignancies worldwide and represents a major public health burden, particularly in developing countries. It primarily arises from hepatic cells, with hepatocellular carcinoma (HCC) being the most prevalent form, followed by cholangiocarcinoma and hepatoblastoma.[1]
- The development of liver cancer is closely associated with chronic liver diseases such as hepatitis B and C viral infections, alcoholic liver disease, and non-alcoholic fatty liver disease. Persistent liver inflammation leads to fibrosis and cirrhosis, creating a favorable environment for malignant transformation through the accumulation of genetic mutations and dysregulation of cellular signaling pathways.[2]
- Oxidative stress and chronic inflammation play crucial roles in hepatocarcinogenesis by inducing DNA damage, lipid peroxidation, and protein

modification, ultimately promoting uncontrolled proliferation of hepatocytes. Liver cancer often remains asymptomatic during early stages, resulting in delayed diagnosis and poor prognosis.[3]

- Common clinical manifestations such as abdominal pain, jaundice, weight loss, and fatigue usually appear in advanced stages of the disease. Diagnostic approaches include imaging techniques such as ultrasound, computed tomography, and magnetic resonance imaging, along with serum biomarkers like alpha-fetoprotein, although early detection remains limited.[4]

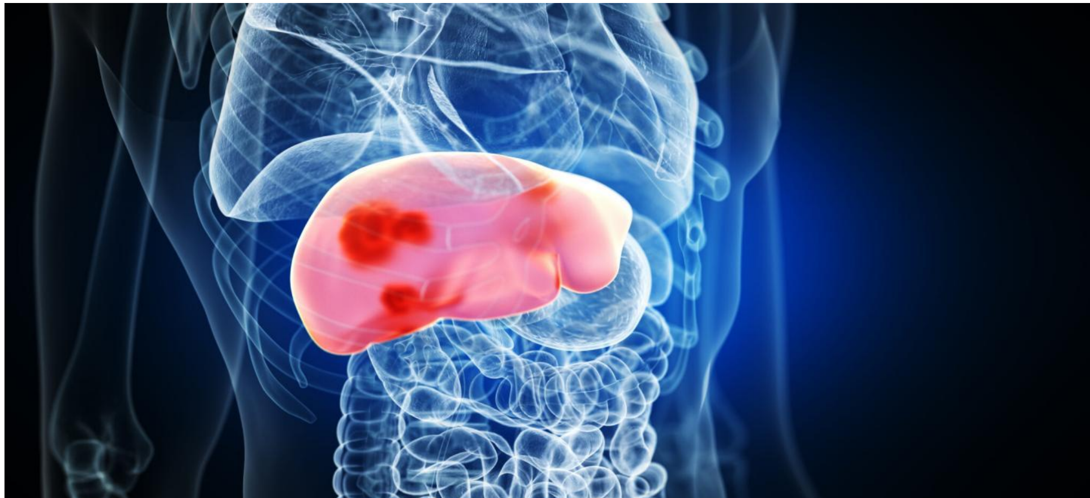

FIG NO: 1 - LIVER CANCER.

CLASSIFICATION OF LIVER CANCER

- Liver cancer is broadly classified into primary liver cancer and secondary (metastatic) liver cancer. Primary liver cancer originates in the liver cells themselves, while secondary liver cancer occurs when cancer spreads to the liver from other organs such as the colon, breast, or lungs.
- Primary liver cancer includes several major types. Hepatocellular carcinoma (HCC) is the most common type and arises from hepatocytes. It is strongly associated with chronic liver diseases such as hepatitis B and C infections, cirrhosis, and alcohol-related liver damage.[5]
- Secondary or metastatic liver cancer is more common than primary liver cancer and occurs when malignant cells from other organs spread to the liver through the bloodstream. This type of liver cancer reflects advanced disease and requires treatment strategies based on the primary tumor origin.

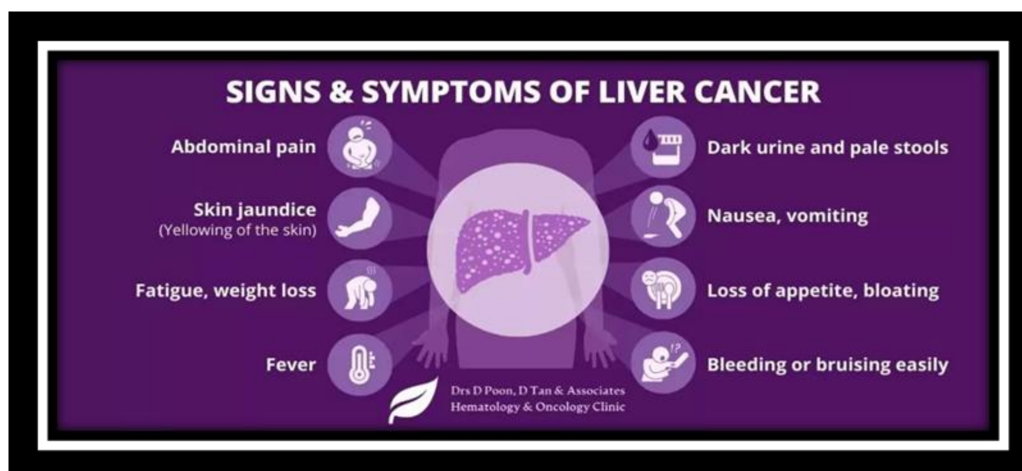

FIG NO: 2 - SIGN AND SYMPTOMS OF LIVER CANCER.

CURRENTLY AVAILABLE DRUGS IN LIVER CANCER

1. Targeted Therapy Drugs

These drugs act on specific molecular pathways involved in tumour growth and angiogenesis.

- **Sorafenib[6]**

A multikinase inhibitor that blocks tumour cell proliferation and blood vessel formation. It is commonly used as first-line therapy in advanced liver cancer.

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- **Lenvatinib**

Inhibits vascular endothelial growth factor (VEGF) receptors and other kinases, reducing tumor angiogenesis. It is an alternative to sorafenib as first-line treatment.[7]

2. Immunotherapy Drugs

These drugs enhance the body's immune response against cancer cells.

- **Nivolumab**

A programmed death-1 (PD-1) inhibitor that helps immune cells recognize and destroy cancer cells.

- **Pembrolizumab**

Another PD-1 inhibitor used in advanced liver cancer, particularly after failure of targeted therapies.

3. Chemotherapy Drugs

Chemotherapy has limited effectiveness in liver cancer but is still used in some cases.

- **Doxorubicin**

One of the most commonly used chemotherapeutic agents in liver cancer.

- **Cisplatin**

Often used in combination regimens or during trans arterial chemoembolization (TACE).[8]

PLANT PROFILE

Tribulus lanuginosus L., commonly known as Gokhru (Woolly Caltrops), is a perennial, prostrate, herbaceous plant belonging to the family Zygophyllaceae. It is widely distributed in the dry, arid, and semi-arid regions of India, particularly in sandy soils, wastelands, roadsides, and open grasslands. The plant is well adapted to harsh climatic conditions and is characterized by its dense woolly (lanuginose) hairs covering the stems and leaves, which help reduce water loss.

A) WHOLE PLANT

- The whole plant of *Tribulus lanuginosus L.,* (Family: Zygophyllaceae) is a prostrate, perennial herb widely found in the arid and semi-arid regions of India. The plant grows close to the ground with spreading branches and is easily recognized by its dense woolly (lanuginose) hairs covering the stem, leaves, and other aerial parts.[9]

B) LEAF

- Leaves are opposite, pinnately compound, consisting of 5–8 pairs of small oblong or elliptic leaflets, all densely pubescent on both surfaces.[10]

FIG NO: 3 - Tribulus Lanuginosus Leaves.

C) FLOWER

The flowers are solitary, axillary, and yellow in color with five petals. They are bisexual and show actinomorphic symmetry.[11]

D) SEED AND FRUIT

- The seeds are small, hard, and enclosed within the nutlets.
- Fruits are hard, spiny schizocarps that break into several sharp-pointed nutlets, aiding in seed dispersal.

E) ROOT

The root system consists of a well-developed tap root with numerous lateral roots, which helps the plant survive in dry and sandy soils.

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BOTANICAL DESCRIPTION

Scientific name: *Tribulus lanuginosus L.*
Family: Zygophyllaceae
Common name: Woolly Caltrop (Indian Tribulus)
Habit: Prostrate or spreading annual herb

Macroscopic (Morphological) Characters

1. Root
The root system is a well-developed tap root with a few lateral branches. Roots are slender, light brown in colour, and help the plant anchor firmly in dry and sandy soils.

2. Stem
Stems are prostrate, spreading along the ground, extensively branched, and covered with dense, soft, woolly (lanuginous) hairs. The stems are green to reddish-brown, cylindrical, and show nodes and internodes clearly.[12]

3. Leaves
Leaflets are oblong to elliptic, small (5–10 mm long), obtuse at the apex, and entire at the margins.
Both surfaces are densely covered with silky, woolly hairs, giving the plant a greyish-green appearance.
Petioles are short and hairy.[13]

4. Flowers
Flowers are solitary, axillary, and yellow in colour. Calyx consists of 5 free sepals, hairy on the outer surface. Flowers are solitary, axillary, and yellow in colour. Corolla has 5 bright yellow, obovate petals.

Androecium consists of 10 stamens arranged in two whorls. Gynoecium is pentacarpellary with a superior ovary.[14]

5. Fruit
The fruit is a schizocarp, breaking into five woody, wedge-shaped mericarps. Each mericarp bears two long, sharp spines and additional smaller spines. Fruits are hard, brown, and characteristically spiny, aiding in dispersal by animals.[15]

6. Seeds
Seeds are small, ovoid, hard, and brownish in colour, one seed per mericarp. Microscopic and Diagnostic Characters (Key Identification Features) Presence of dense lanuginous (woolly) trichomes on stem and leaves Paripinnate leaves with small opposite leaflets Yellow axillary flowers Distinctive spiny schizocarp fruit.

Major Phytochemical Classes Identified

Phytochemical screenings of *T. lanuginosus*/*T. terrestris* extracts reveal the presence of the following groups of compounds

1. Saponins
2. Flavonoids & Phenolics
3. Alkaloids
4. Glycosides
5. Tannins & Phenols
6. Other Metabolites

PHARMACOLOGICAL USES OF *Tribulus lanuginosus*

1. Hepatoprotective Activity

- Protects liver cells from chemical-induced damage.
- Antioxidant and membrane-stabilizing effects contribute to liver protection.

2. Anti-inflammatory Activity

- Reduces inflammation by inhibiting prostaglandin and cytokine release.
- Beneficial in inflammatory disorders and tissue swelling.

3. Analgesic Activity

- Exhibits mild to moderate pain-relieving effects.
- Associated with reduction of inflammatory mediators.

4. Antimicrobial Activity

- Shows activity against selected bacterial and fungal strains.
- Supports traditional use in infections and wound healing.

MATERIALS AND METHODS

SELECTION AND AUTHENTICATION OF PLANT MATERIAL

We have selected the *Tribulus lanuginosus* plant, to study the in-vitro hepatoprotective activity. The taxonomic identities of the plant were confirmed by Botanical survey of india, Coimbatore (590) The herbarium was stored in our pharmacology department.

COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

Fresh leaves of *Tribulus lanuginosus* will be collected, washed, and shade-dried. The dried leaves will be coarsely powdered using a mechanical grinder and stored in airtight containers. This powdered material will be used for preparation of ethanolic and aqueous extracts for further studies.

PLANT EXTRACTION

About 100 g of the dried powder was transferred into a clean, dry conical flask. ethanolic solvent system. Ethanol was added in sufficient quantity to completely immerse the plant material. The flask was tightly closed and kept for 72 hours at room temperature for cold maceration, with intermittent shaking at regular intervals to ensure proper solvent penetration and extraction of phytoconstituents.

After maceration, the extract was filtered through muslin cloth followed by Whatman No.1 filter paper to remove plant debris. The marc was re-macerated with fresh solvent to ensure maximum extraction, and the filtrates were pooled.

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Percentage Yield (% w/w) = $\frac{\text{Weight of dried extract obtained (g)}}{\text{Weight of dried plant material used (g)}} \times 100$

PRELIMINARY PHYTOCHEMICAL SCREENING

Tribulus lanuginosus Extract

1. Preparation of Test Solution[16]

- Weigh 1 g of dried powdered extract (ethanolic or aqueous).
- Dissolve in 10 mL of respective solvent.
- Filter and use the filtrate for phytochemical tests.

Test for Alkaloids

a) Mayer's Test

- Add a few drops of Mayer's reagent to 2 mL of extract.[17]
- **Observation:** Cream or white precipitate
- **Inference:** Presence of alkaloids

b) Dragendorff's Test

- Add Dragendorff's reagent to the extract.[18]
- **Observation:** Orange or reddish-brown precipitate
- **Inference:** presence of alkaloids

C) Wagner's Test

- Add wagner's reagent to the extract.[19]
- **Observation:** Brown or reddish-brown precipitate
- **Inference**: Presence of alkaloids

Test for Glycosides

a) Liebermann's Test

- Add 2.0 ml of chloroform and concentrated acetic acid to the plant extract in the ice bath. Add few drops of concentrated sulfuric acid (H_2SO_4).[20]
- **Observation:** violet to green
- **Inference:** presence of glycosides

Test for Carbohydrates

a) Molisch's Test

- Add Molisch reagent and conc. H_2SO_4 carefully.
- **Observation:** Violet ring
- **Inference:** presence of Carbohydrates

Test for Saponins

a) Foam Test

- Shake 2 mL extract with 5 mL distilled water vigorously.
- **Observation:** Persistent foam (≥1 cm)
- **Inference:** presence of Saponins

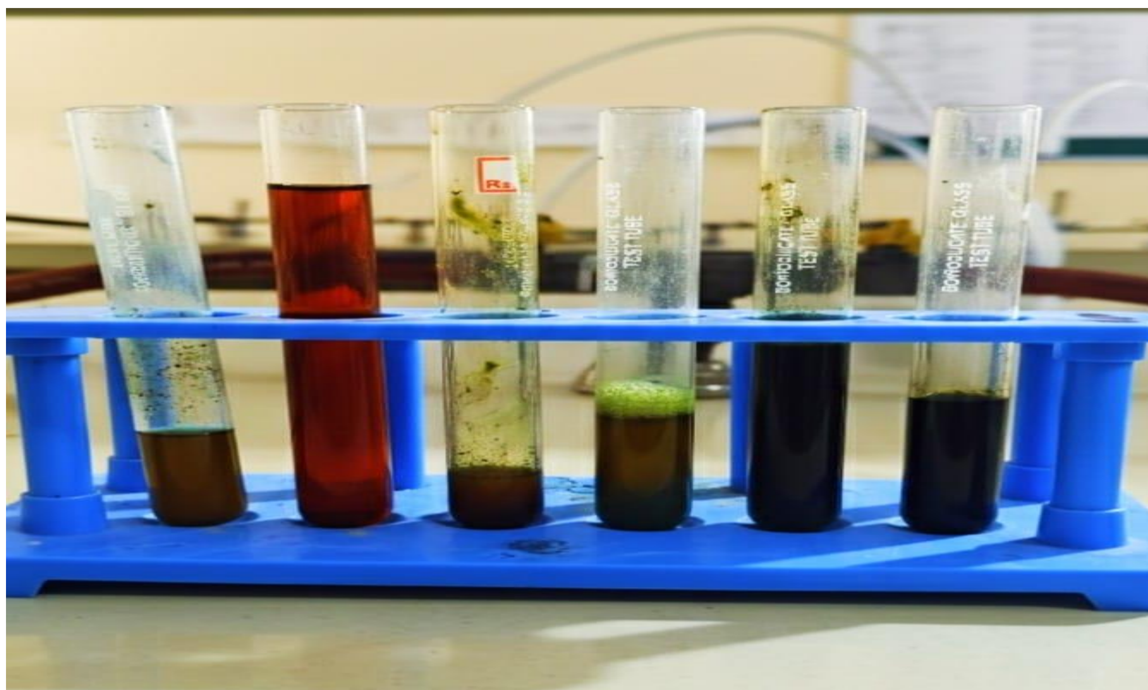

FIG NO: 4 - PHYTOCHEMICAL TEST FOR *Tribulus lanuginosus.*

In - vitro Anticancer activity (MTT Assay)

- For the Hepto productivity of HepG2 Cell Line was used to study the anticancer activity of the sample. The cell Line were purchased from National centre for cell Science, Pune, India and has been maintained further in centre for Bioscience and Nano science Research laboratory Eachanari, Coimbatore, Tamil Nadu, India. After obtaining, the Cell Line was subcultured to RPMI medium with the addition of sodium carbonate, glucose and BSA (10%). After adding all the chemicals in T flask, the cells was incubated in CO_2 incubator with the pH of 7 to 7.5, temperature 37^0C, humidity 70-80% for 24-72 hrs. After incubation the growth of the cell line

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was confirmed by viewing under inverted microscope and used for further study.[21]

- For the MTT assay, cells were again seeded in 96-well plates and allowed to adhere for 24 hrs at $37^{O}C$ in 5% CO_2 and 70-80% of humidity. Cell line with sample in different concentration (10 to 50ul) along with blank (DMSO), control (CellLine), and standard drug (Doxorubicin-12.5µg) was incubated for 24 hrs. After incubation the cells were washed with DMSO, and trypsin after washing 20µl of MTT dye was added to each well, after slight mixing the plates were incubated for 24hrs at $37^{o}C$ in Co_2 incubator.[57] The reaction mixture was then carefully taken out and formazan crystals were solubilized by adding 100 ml of DMSO to each well and mixed thoroughly. After 24hrs the absorbance of purple color were read at 570 nm using 96 well plate ELISA reader (Robonik, India) After taking the % of cell viability were calculated by following formula.[22]

% cell viability= [Mean abs of treated cells/Mean abs of Untreated cells] x 100

RESULTS AND DISCUSSION

PERCENTAGE YIELDS OF LEAVES EXTRACT

About 100 g of the dried powder was transferred into a clean, dry conical flask. A ethanolic solvent system. Ethanol was added in sufficient quantity to completely immerse the plant material. The flask was tightly closed and kept for 72 hours at room temperature for cold maceration, with intermittent shaking at regular intervals to ensure proper solvent penetration and extraction of phytoconstituents.

TABLE NO: 1 - % YIELD OF LEAVE EXTRACTS OF *Tribulus lanuginosus*.

S.NO	WEIGHT OF SAMPLE (gm)	SOLVENT	IMMERSION TIME	WEIGHT OF CRUDE EXTRACT (gm)	% YIELD
1)	100	ETHANOL	5 DAYS	14.4	14.4%

PHYTOCHEMICAL SCREENING

TABLE NO: 2 – PHYTOCHEMICAL ANALYSIS OF ETHANOLIC LEAVE EXTRACTS.

S.NO	COMPOUND	ETHANOLIC EXTRACT
	ALKALOIDS	
1.	MAYER 'S TEST	+ VE
2.	DRAGENDORFF 'S TEST	+ VE
3.	WAGNER 'S TEST	+ VE
SAPONINS		
4.	FOAM TEST	+ VE
GLYCOSIDES		
5.	LIBERMANN 'S TEST	+ VE
CARBOHYDRATES		
6.	MOLISH 'S TEST	+ VE

IN-VITRO ANTI-CANCER ACTIVITY

The in-vitro anticancer evaluation revealed that the test extract exhibited a significant, dose-dependent cytotoxic effect against the selected cancer cell line. As the concentration increased, a gradual reduction in cell viability was observed, indicating effective inhibition of cancer cell proliferation. The extract demonstrated notable morphological changes such as cell shrinkage, rounding, and loss of adherence, which are characteristic features of apoptosis. The calculated IC_{50} value confirmed moderate to strong anticancer potential when compared with the standard anticancer drug. Overall, the results suggest that the extract possesses promising in-vitro anticancer activity and supports its potential for further mechanistic and in-vivo studies.[23]

OBSERVATION

The in-vitro anticancer study showed a clear, concentration-dependent cytotoxic effect of the test extract on the selected cancer cell line. As the concentration of the extract increased, a gradual reduction in cell viability was observed when compared with the untreated control cells.[24] Morphological changes such as cell shrinkage, rounding, loss of adherence, and membrane blebbing were evident under microscopic examination, indicating induction of cell death. At lower concentrations, cells exhibited mild growth inhibition, whereas higher concentrations produced significant suppression of cell proliferation. The IC_{50} value indicated moderate to good anticancer potential of the extract. Overall, the observations suggest that the extract possesses promising in-vitro anticancer activity and may act by inhibiting cancer cell growth and inducing cytotoxic effects.[25]

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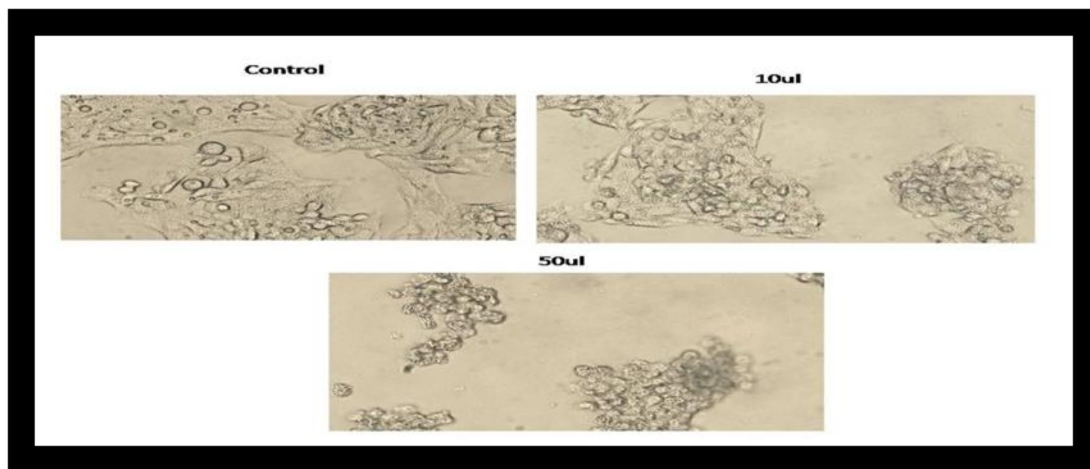

FIG NO: 6 – MICROSCOPICAL STRUCTURE OF ACTIVITY.

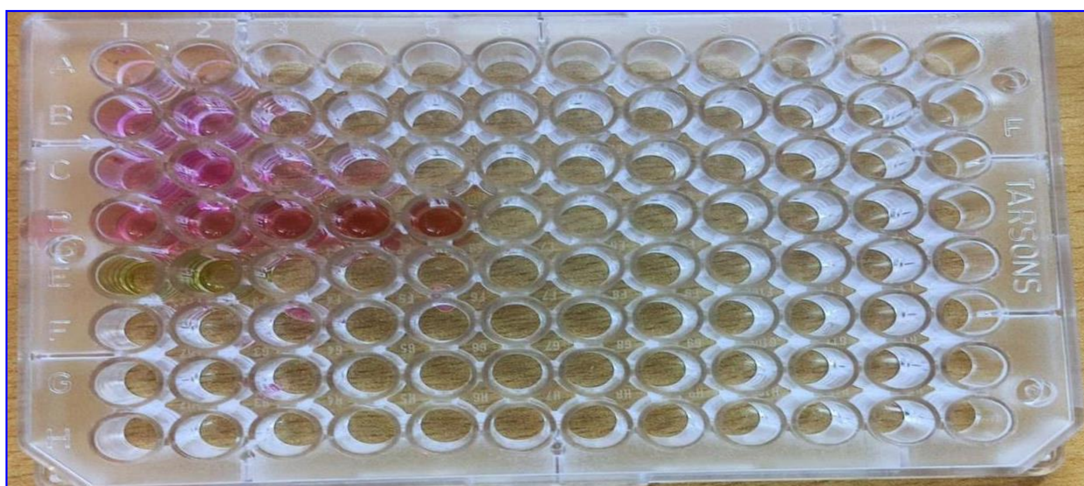

FIG NO: 7 - 96 WELL PLATE.

OBSERVATION TABLE

TABLE NO: 3 – ANTICANCER ACTIVITY OF ETHANOLIC EXTRACTS OF *Tribulus lanuginosus*.

S.NO	EXTRACT	CONCENTRATION (mg/ml) (TEST)	INHIBITION %	CELL VIABILITY % (100 – INHIBITON)
1.	ETHANOLIC EXTRACT	10 mg/ml	3.980 %	96.020 %
		20 mg/ml	16.242 %	83.758 %
		30 mg/ml	25.00 %	75.00 %
		40 mg/ml	48.248 %	51.752 %
		50 mg/ml	53.184 %	46.816 %

IC50 CALCULATION

IC50 stands for half maximal inhibitory concentration. It is a measure of the potency of a substance in inhibiting a specific biological or biochemical function, indicating ho w much of a particular inhibitory substance (e.g., drug) is needed toinhibit a biological process by 50%. In simpler terms, a lower IC50 value indicates a more potent inhibit or, meaning less of the substance is required to achieve t he desired inhibitory effect.[26]

1. IDENTIFY AND SURROUNDING POINTS

- At concentration 40, inhibition is 48.248 %
- At concentration 50, inhibition is 53.184%
- Since 50% falls between these two, the IC50 is between 40 and 50.

2. PLUG VALUES INTO THE FORMULA

$C_{50} = x_1 + [(50 - y1) / (y_2 - y_1)] \times (x_2 - x_1)$

3. CALCULATE THE RATIO[27]

- Difference to target: 50 – 48.248 = 1.752
- Total inhibition gap: 53.184 – 48.248 =4.936
- Ratio: 1.752 / 4.936 = 0.3549

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4. FINAL RESULT

$C_{50} = 40 + [(50 - 48.248) / (53.184 - 48.248)] \times (50 - 40)$

$IC50 = 40 + (0.3549 \times 10)$

$IC50 = 43.55$ units

GRAPHICAL REPRESENTATION

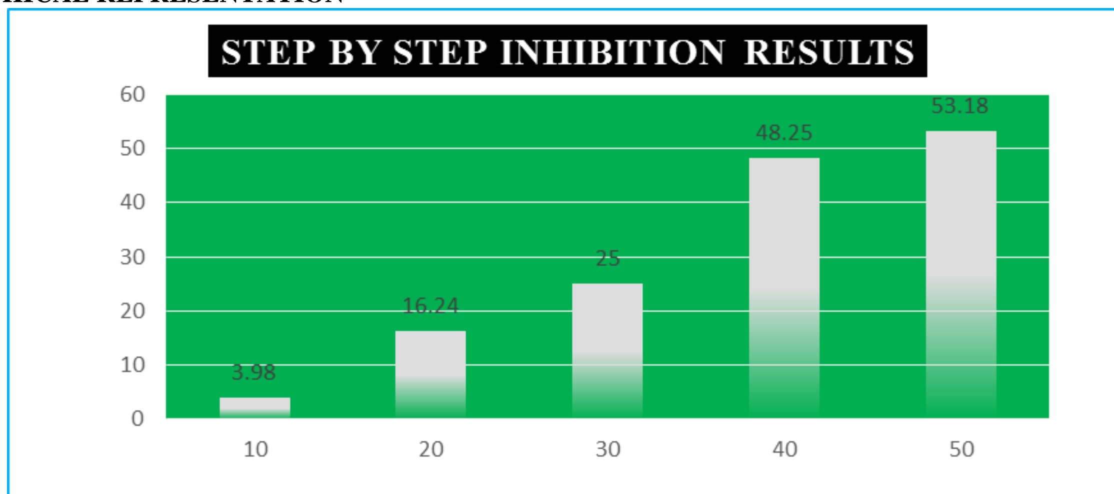

FIG NO: 8 – CONCENTRATION VS INHIBITION %

SUMMARY AND CONCLUSION

Tribulus lanuginosus ethanolic extract showed strong in vitro anticancer activity against the chosen cancer cell line, as evidenced by a dose-dependent decrease in cell viability and powerful cytotoxic potential. In particular, treated cancer cells showed significant morphological alterations, such as cell shrinkage, rounding, and loss of adherence, while retaining a significant IC•¹ value that indicates moderate to strong effectiveness. The extract effects were concentration sensitive, with stronger cytotoxic effects at higher concentrations and partial growth inhibition at lower dosages. Additionally, when compared to normal cell controls, the extract demonstrated specific toxicity toward cancer cells, confirming *Tribulus lanuginosus* traditional medical significance as a possible anticancer agent.in this study finds that the extract has promise anticancer characteristics, but more in-vivo and molecular-level research is needed to properly comprehend its potential for future therapeutic use.

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