

In Vitro Screening of Anticancer Activity and Phytochemical of *Connarus Wightii* Medicinal Plant

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Abstract

Due to the rising death rate from cancer worldwide, it is one of the biggest worries. Globally, medicinal plants have a significant impact on people's health. According to recent research, on the phytochemical analysis of medicinal plant in southern Tamilnadu, particularly in the southwest portion of the Kanayakumari district. Thus, the primary goal of this study was to identify the phytochemical compounds, antioxidant properties and anti-cancer properties of medicinal plant *connarus wightii*. An analysis was conducted on the phytochemical make up of these extracts (chloroform, ethanol, and methanol). Additionally, the extracts from these plants were assessed for their anti-cancer properties. The most prevalent phytochemical components in the plants under investigation were found to be phenols, flavonoids, saponins, glycosides etc. According to recent research, plant extracts have anticancer action of *connarus wightii*. As the result of anticancer investigation using the MTT assay, the test samples, chloroform, ethanol, and methanol extracts of *connarus wightii*, were strongly cytotoxic to human liver cancer (HepG2) cells with low IC₅₀ values of 99.91, 158.12, and 47.42 µg/ml, respectively. They had an IC₅₀ of less than 10 µg/ml against the cancer cell lines was investigated. This low IC₅₀ suggests that crude extracts have potential as an anticancer drug. The significance of these identified plant species as a source of anticancer drugs was therefore emphasised by the current investigation.

Keywords: phytochemical screeeing, *connarus wightii*, antioxidant properties, anti-cancer properties

Introduction

Cancer is a condition in which the body's own cells multiply and spread abnormally without control. In industrialised countries, it is one of the leading causes of death. About one-fourth of all deaths worldwide are caused by cancer, with lung and bowel cancer making up the biggest category and breast and prostate cancer coming in second and third, respectively (Rantg et al., 2010). People have employed medicinal herbs for centuries. Secondary metabolites, the chemical substances created by plants, are recognised to be crucial to their biological processes. Secondary metabolites present in the herbal plants that play vital role in the defensive mechanism of cancer cells. Plant secondary metabolites are essential for maintaining human health and are the building blocks of many pharmaceuticals. Indeed, Plant secondary metabolites account for about 25% of all currently available medications. Getting access to natural resources, particularly medicinal plants, is one of the ongoing hurdles for the drug research and pharmaceutical industry (Kessler et al., 2018).

Cancer is currently the most lethal and common disease. In 2018, there were 9.6 million cancer-related deaths and 18.1 million new cases. Cancer has 36 distinct forms, with men and women being the primary populations affected by colorectal, liver, lung, stomach, and prostate cancers, respectively (Bray et al., 2018). Both traditional and extremely contemporary methods are used to treat cancer. Chemotherapy, radiation treatment, or surgery are just a few of the methods utilised to treat cancer. But each of them has certain drawbacks. The majority of cancer medications presently in use have adverse effects, and not all cancers react to therapy the same way. Therefore,

new methods or chemicals are needed for the treatment of cancer (Huang et al. 2011). Plant-based organic compounds called phytochemicals, including phenolics, are non-nutritive bioactive molecules that have been associated with anti-cancer characteristics in a range of plant-based meals (Tapsell et al., 2006). Moreover, these compounds' diverse anti-inflammatory and antioxidant qualities help to lessen the harm that free and active oxygen causes to the body (Amira et al., 2012). This is mostly because plants have a variety of chemicals, such as potent antioxidants called flavonoids and phenyl propanoids (Cai et al., 2004).

According to Krishna et al. 2021, based on estimates of 2 million diagnoses and 1.8 million deaths, lung cancer is the primary cause of cancer incidence and mortality worldwide. Following prostate and breast cancer, respectively, neoplasms of the lungs are the second most prevalent cancer diagnoses in both sexes. Lung cancer incidence is rising worldwide as a result of growing tobacco access and industrialization in emerging countries. Due to its resistance to the majority of therapeutic therapies and evidence of metastases inside lymph nodes, lung cancer is also the most lethal kind of cancer (Burris, 2009; Benlloch et al., 2009). Every year, more than 10 million new cases of cancer are scrutinized, according to the World Health Organization (WHO), and statistical trends predict that this number will double in the decades. Cancer is the second prevalent cause of mortality worldwide, consumed the lives of 10 million people in 2020 (Pandey et al., 2009). Cancer diagnoses are expected to rise by 50% from 14 million to 21 million, while cancer deaths are expected to rise by 60% from 8 million to 13 million. Lung cancer (1.69 million deaths), stomach cancer (754000 deaths), colorectal cancer (774000 deaths), liver cancer (788000 deaths), and breast cancer (571000 deaths) are the most prevalent kinds of cancer death. The number of cancer cases is predicted to climb by around 70% in the future decades, with the majority of deaths occurring in low and middle-income nations (Boccardo et al., 2011). Certain lifestyle choices, customs, infectious agents, alcohol consumption, and environmental toxins are some of the mostwell-known reasons for this illness (Kuper et al., 2011).

As part of the research effort to find anticancer medications based on herbal treatments, Non-communicable illnesses like cancer are beginning to cause significant public health issues in India. Many individuals are increasingly turning to herbal remedies and phytonutrients, often known as nutraceuticals, for the treatment of a variety of health issues in various national healthcare systems, as the usage of these products continues to grow fast around the world (WHO, 2004).

In order to identify the phytochemical elements of the plants that are responsible for a certain bioactivity, phytochemical screening of plant extracts is therefore essential, especially those that have been used in traditional medicine. The current study's objective was to evaluate the *connarus wightii* herbs' in vitro cytotoxic abilities utilising the human liver cancer (HepG2) cancer cell line. The herbs were gathered from southern Taminadu, Tholayavattam. This was done as a part of our research on substances derived from plants that have anticancer effects.

MATERIALS AND METHODS

Collection of Medicinal Plant Sample

In this study, fresh *connarus wightii*, a therapeutic herbal plant, was collected from several sites around Tholayavattam in the southern Tamilnadu Kanayakumari district. The plant materials were shade dried in order to get properly dried and prepared for grinding. A taxonomist from Tamil Nadu Agricultural University (TNAU), Southern Circle, Coimbatore, verified the plant samples. The mechanical blender was used to fully grind the dried plant parts into a fine powder, which was then placed in labelled sealed containers.

Solvent Extraction for *Connarus wightii*

The Soxhlet extraction process was used to create crude plant extract. A consistent 20gm of powdered plant material was placed in a thimble, and 250ml of various solvents were extracted from the selected medicinal plant. Three different solvent Methanol, chloroform and ethanol were used to extract the plant compounds. The extraction process continues for five hours or until the solvent in the extractor's syphon tube becomes colorless. The extract was then placed in a beaker and cooked on a hot plate at 30 to 40 degrees Celsius until the solvent had evaporated. Dried extract was kept in refrigerator at 4°C for their future use.

Qualitative Phytochemical Analysis of *Connarus wightii*

Connarus wightii leaf extracts were extracted using several solvents, including chloroform, ethanol, and methanol, and their phytochemical composition was analysed qualitatively using methods described by Chitravadivu et al. (2009) and Sakamoto et al. (2018).

Detection of Carboxylic Acid

Plant extract (1 ml) is combined with sodium bicarbonate solution (2 ml). Carboxylic acid is present when test-brisk effervescence indicates colour changes occur.

Detection of Tannins

Before being heated for 5–6 minutes, 2-3 ml of 10% HCL are mixed with 2 ml of plant extract. As the results the formation of a red colour indicates the presence of tannins.

Detection of Steroids

0.5 ml of the extract was mixed with 5 ml of chloroform and an equal volume of concentrated H_2SO_4 . The creation of red colour in the top layer and yellow with green colour in the bottom layer indicates the presence of steroids.

Detection of Flavanoids

0.5 ml of the extract was mixed with 1 ml of concentrated H_2SO_4 and 4 ml of 1% ammonia. Flavanoids are indicated by the appearance of a yellow colour.

Detection of Glycosides (Born- Trageru's Test)

When 2 ml of hydrolysate and 3 ml of chloroform are combined and aggressively shaken, the chloroform layer separates. Then 10% ammonia solution was added. A pink coloration that develops is indicated the present of glycosides.

Detection of Proteins (Bradford Method)

500 ml of plant extract was mixed with 5 ml of the Bradford reagent, and the combination was incubated for 10 to 15 minutes in the dark before the OD at 575 nm was measured.

Detection of Phenols (Ferric Chloride Test)

50 mg of the extract was diluted with 5 ml of distilled water and a few drops of a 5% ferric chloride solution. A dark green coloration indicated the present of phenols.

Saponin Test

50 mg of plant extract was combined with 20 ml of distilled water, and the mixture was agitated vigorously for 15 minutes. A 2 cm layer of foam was produced to detect the presence of saponins.

Test for Alkaloids - Mayer's test

A few ml of plant sample extract receives two drops of Mayer's reagent along the test tube's sidewalls. When a precipitate appears white and creamy, alkaloids are present.

Saponification test

When 1 or 2 ml of 10 N sodium hydroxide combined with 2 ml of extract were heated for 2 minutes, soap or fat will form as a result. Oils and fats are present when a solution is soupy. This test's application to the extracts under examination revealed that there were no oils or fats present.

Gum Test

In 2 ml of distilled water, 100 mg of plant extract were diluted. Pure alcohol in 2 ml, constantly stirred. Cloudy precipitation with a white colour forms indicates the presence of gums and mucus.

Detection of flavanoglycoside

The 50 mg of plant extract was dissolved in 5 ml of ethanol. Added a few drops of magnesium sulphate and concentrated HCL. A pink colour appears when flavanoglycoside is present, suggesting its presence.

Detection of Carbohydrates

For 2 minutes, 0.5 ml of extract was heated with Benedict reagent. Precipitate formation and colour changes both happen. It acts as an indicator of the presence of carbohydrates.

Detection of resins

0.5 ml of plant extract is combined with 3 ml of the CuSO₄ solution. Resins are present when green precipitate develops after 1-2 minutes of shaking.

Biuret test

2 ml of extract and 1 drop of a 2% CuSO₄ solution. Add 2 to 3 sodium hydroxide pellets and 1 millilitre of 95% ethanol together. Testing is positive when pink coloration forms.

QUANTITATIVE PHYTOCHEMICAL SCREENING OF *CONNARUS WIGHTII*

Quantitative Estimation of Proteins

The Lowry et al. (1951) method was used to measure protein content. A mixture of 1 ml of sample, 0.5 ml of sodium hydroxide at 0.1 N, and 5 ml of alkaline copper reagent was added. For 30 minutes, the mixture was incubated at room temperature. Folin-Ciocalteu reagent was then added, and the mixture was again incubated for 10 minutes at room temperature. At 660 nm, the absorbance was measured against a blank for the reaction. Using BSA (bovine serum albumin) as a reference material, the assay was compared to BSA equivalents.

ANTIOXIDANT PROPERTIES OF *CONNARUS WIGHTII*

DPPH Radical Scavenging Activity

In studies on natural product antioxidants, the DPPH test is frequently used. The simple nature and sensitivity of this approach is one of the reasons. The idea behind this test is that an antioxidant is a hydrogen donor. It counts substances that scavenge radicals. One of the few stable and easily obtained organic nitrogen radicals is DPPH. The reduction of DPPH in test samples reflects the antioxidant action. Due to its ease and precision, monitoring DPPH with a UV spectrometer has become the most popular technique. At 517 nm (purple), DPPH has a significant absorption maximum. When hydrogen from an antioxidant is absorbed, the colour changes from purple to yellow and DPPH is then produced. In terms of the quantity of hydrogen atoms absorbed, this reaction is stoichiometric. As a result, the reduction in UV absorption at 517 nm makes it simple to assess the antioxidant impact.

The free radical scavenging activity of the fractions was measured in vitro by 2,2' - diphenyl-1-picrylhydrazyl (DPPH) assay according to the standard method (Williams et al., 1995). The stock solution was prepared by dissolving 24 mg DPPH with 100 ml of ethanol stored at 20°C until required. The working solution was obtained by diluting DPPH solution with ethanol and 3 ml aliquot of this solution was mixed with 1 ml of sample at various concentrations (10, 50, 100, 250 and 500 µg/ml). The reaction mixture was shaken well and incubated in the dark for 15 minutes at room temperature. Then the absorbance was taken at 517 nm. The control was prepared without any sample and scavenging activity was estimated based on the percentage of DPPH radical scavenging as the following equation.

$$\text{Percentage of inhibition} = \left[\frac{(\text{control OD} - \text{sample OD})}{(\text{control OD})} \right] \times 100$$

Ferric Reducing Antioxidant Potential (FRAP) Assay

A modified version of the FRAP assay was used to assess the ferric reducing capacity of plant extracts. This method is based on the reduction, at low pH, of a colorless ferric complex (Fe³⁺-tripyridyltriazine) to a blue-colored ferrous complex (Fe²⁺-tripyridyltriazine) by the action of electron-donating antioxidants. The decrease in absorbance at 593 nm is used to measure the reduction. The working FRAP reagent was prepared daily by mixing

10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM hydrochloric acid and with 1 volume of 20 mM ferric chloride. A standard curve was prepared using various concentrations of $\text{FeSO}_4 \times 7\text{H}_2\text{O}$.

All solutions were used on the day of preparation. One hundred microliters of sample solutions and 300 μl of deionized water were added to 3 ml of freshly prepared FRAP reagent. The reaction mixture was incubated for 30 min at 37°C in a water bath. Then, the absorbance of the samples was measured at 593 nm. A sample blank reading using acetate buffer was also taken. The difference between sample absorbance and blank absorbance was calculated and used to calculate the FRAP value. In this assay, the reducing capacity of the plant extracts tested was calculated with reference to the reaction signal given by a Fe^{2+} solution. FRAP values were expressed as $\text{mmol Fe}^{2+}/\text{g}$ of sample. All measurements were done in triplicate.

$$\text{Percentage of inhibition} = \left[\frac{(\text{control OD} - \text{sample OD})}{(\text{control OD})} \right] \times 100$$

ANTICANCER ACTIVITY OF *CONNARUS WIGHTII* EXTRACT

Cell Culture and Maintenance of human liver cancer (HepG2) cells

Human liver cancer (HepG2) cells were bought from NCCS, Pune and cultivated in liquid medium (DMEM) supplemented with 10% Foetal Bovine Serum (FBS), 100 $\mu\text{g}/\text{ml}$ penicillin, and 100 g/ml streptomycin, and kept at a temperature of 37°C in a 5% CO_2 environment.

MTT Assay of human liver cancer (HepG2) cells

The samples were tested for *in vitro* cytotoxicity, using HepG2 cells by MTT assay. Briefly, the cultured HepG2 cells were harvested by trypsinization, pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 μL) into the 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C . The wells were washed with sterile PBS and treated with various concentrations of the samples in a serum free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO_2 incubator for 24 h. After the incubation period, MTT (20 μL of 5 mg/ml) was added into each well and the cells incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 μL) were aspirated off the wells and washed with 1X PBS (200 μl). Furthermore, to dissolve formazan crystals, DMSO (100 μL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a micro plate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC_{50} value was calculated using Graph Pad Prism 6.0 software (USA).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples}}{\text{Mean OD of control group}} \times 100$$

The entire plate was seen in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) every 24 hours for up to 72 hours, and microscopic observations were recorded as pictures. Any visible changes in cell shape, such as rounding or shrinkage of cells, granulation, and vacuolization in the cytoplasm, were deemed cytotoxicity indications.

RESULT AND DISCUSSION

The *Connarus wightii* plant was chosen for this study's emphasis on biological characteristics. *Connarus wightii* extracts are made from the leaves by extracting them using ethanol, methanol, and chloroform, three distinct solvents. Various Photochemical analysis existences and their *Connarus wightii* have potential as an anti-oxidant and anti-cancer activity of extracts was evaluated. To identify their bioactive components, chloroform, methanol, and ethanol extracts of *Connarus wightii* leaves were qualitatively screened for phytochemical substances. Phytochemical characterization and illustrations of different the solvent extracts from the *Connarus wightii* were carried out and the results are tabulated in Table-1 Plant extracts were subjected to phytochemical analysis, which identified components that have been linked to both physiological and therapeutic effects. Following a

phytochemical analysis, it was discovered that the plant extracts included Resins, Carboxylic acid, phenols, tannins, flavonoids, saponins, carbohydrates, steroids, gum, glycosides, and alkaloids. The phytochemical analysis of the chloroform extracts indicated the presence of resins, carboxylic acid, steroids, flavonoids, carbohydrates, protein, and gum. The presence of resins, carboxylic acid, steroids, carbohydrate, protein, and phenol was clearly visible in the ethanol extracts of *C. wightii*. Resins, carboxylic acid, steroids, flavonoids, carbohydrates, and protein were all present in the methanol extracts. According to the quantitative measurement of protein, methanol (0.53 g/g) had the highest concentration of protein, followed by ethanol (0.51 g/g) and chloroform (0.35 g/g) (Table - 1). According to Mohan et al. (2014) Similar results in these therapeutic herbs. According to Phytochemical analysis, compounds like alkaloids and saponins are known to be useful for treating syphilis and other venereal disorders. He had previously observed that saponins contain antibiotic characteristics, which assist the body fight infections and microbial invasion. Additionally, it is employed as a mild detergent and in intracellular histochemistry labelling to provide antibody access to intracellular proteins, which have been linked to weight reduction, hyperglycemia, antioxidant, anticancer, and anti-inflammatory effects in *C. wightii*.

Table-1. Phytochemical screening of *Connarus wightii*

Phytocompounds	Solvent extracts		
	Chloroform	Ethanol	Methanol
Resins	+	+	+
Carboxylic acid	+	+	+
Tannins	-	-	-
Steroids	+	+	+
Flavonoid	+	-	+
Carbohydrates	+	+	+
Glycosides	-	-	-
Protein	0.35 µg/g	0.51 µg/g	0.53 µg/g
Phenol	-	+	-
Biuret	-	-	-
Saponin	-	-	-
Gum	+	-	-
Flavanoglycosides	-	-	-
Alkaloids	-	-	-

Note: + ve indicates positive result, whereas – ve indicates negative result

ANTIOXIDANT PROPERTIES

DPPH Radical Scavenging Assay

Antioxidants, which act as reducing agents and minimise oxidative damage to biological structures by giving free radicals electrons and passivating them, are strongly linked to oxidative damage. Free radicals are created when oxygen interacts with certain molecules, and once they are established, their main threat stems from the harm they may do when they interact with vital biological elements including DNA, proteins, and the cell membrane (Dröge.,2002). Antioxidants interact with these free radicals in a way that finally neutralizes them before harm is done (Hallowell., 2012). Many of the chemicals that plants produced by the medicinal plants as a secondary metabolites that have an antioxidant properties. In order to learn more about extracts of *C. wightii* capacity to scavenge free radicals in vitro, the current investigation was conducted. The Table -2 shows the outcomes of the

DPPH radical scavenging experiment performed on chloroform, ethanol, and methanol extracts of *C. wightii* leaves. Values that are equivalent to normal ascorbic acid are also indicated in Table -2. Chloroform and methanol extracts from the leaves of *C. wightii* were much more effective in scavenging free radicals than the conventional ascorbic acid and ethanol extracts (IC₅₀: 44.93 g/ml and 59.00 g/ml, respectively). The concentration-dependent DPPH experiment utilising conventional ascorbic acid and *Connarus wightii* extracts in chloroform, ethanol, and methanol is shown in Fig-1. Stable free-radical molecules make up the crystalline powder known as DPPH, which is black in colour. Most importantly, it is a well-known radical and a typical antioxidant test. When neutralised and transformed into DPPH-H, the DPPH radical loses its colour and turns light yellow or colourless (Jagetia et al., 2003).

The results of the DPPH radical scavenging assay of chloroform, ethanol and methanol extracts of *Connarus wightii* leaves are presented in Table -2. In Table -2, values that correspond to standard ascorbic acid are also mentioned. Among the extracts of *Connarus wightii* leaves, chloroform and methanol showed significant dose-dependent radical scavenging activity (IC₅₀- 44.93 µg/ml and 59.00 µg/ml respectively) than the standard, ascorbic acid (IC₅₀ - 93.935 µg/ml) and ethanol extract (IC₅₀- 113.3 µg/ml).

Table-2 Percentage of inhibition of *Connarus wightii* leaf extracts at various concentrations by DPPH assay

Sample concentration (µg/ml)	Percentage of inhibition (%)			
	Chloroform	Ethanol	Methanol	Ascorbic acid (Standard)
10	15.07±1.86	40.72±0.93	56.06±4.95	15.921 ± 0.051
50	55.62±7.23	44.44±0.67	68.81±3.67	48.219 ± 0.096
100	74.59±7.02	62.09±5.46	74.69±0.53	64.247 ± 0.701
250	82.51±0.82	72.62±2.98	78.83±2.41	86.152 ± 0.026
500	85.95±0.99	82.76±3.14	83.14±1.29	97.997 ± 0.16
IC ₅₀	44.93	113.3	59.00	93.935

FRAP (Ferric Reducing Antioxidant Potential Assay)

This study evaluated the reducing capacity of MEs of different medicinal plants using the Fe³⁺ to Fe²⁺ reduction test. Here, the solution's colour changed from yellow to a light green or blue due to the antioxidant activity of the samples. Every sample of medicinal plants showed evidence of the presence of many antioxidants, including phenolic acid and flavonoids, in significant amounts. The FRAP test may be used to track an antioxidant's ability to donate electrons to a reducing agent, such as antioxidants, causing the Fe³⁺-ferricyanide complex to reduce and produce ferrous (Fe²⁺) ions and a chromogenic complex (Soobrattee et al., 2003). The quantity of Fe²⁺ in the mixture was determined by measuring the absorbance of the blue-green coloured solution of samples that resulted from the process at 593 nm. The varied *Connarus wightii* leaf solvent extracts were examined for FRAP activity (Table -3). In the presence of an antioxidant, methanol extract exhibited the greatest level of inhibition (IC₅₀ value: 109.604 µg/ml), followed by extracts from ethanol (IC₅₀: 120.036 µg/ml) and chloroform (IC₅₀: 177.561 µg/ml). Standard drug demonstrated more inhibitory efficacy than solvent extracts, with an IC₅₀ value of 67.78 µg/ml.

Table-3. Percentage of inhibition of *C. wightii* leaf extracts at various concentrations by FRAP assay

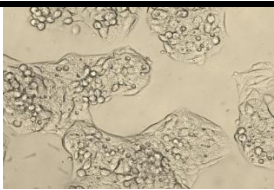
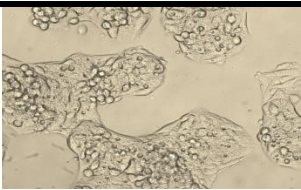
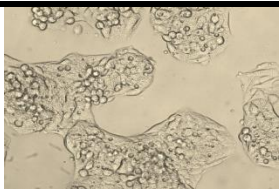
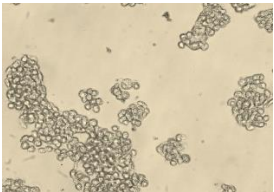
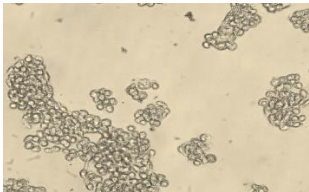
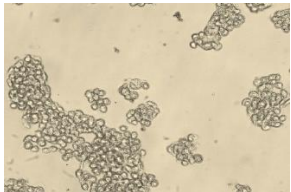
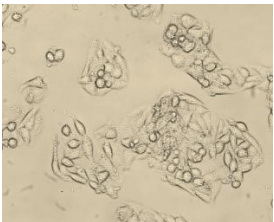
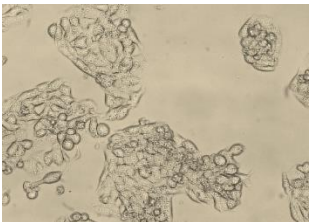
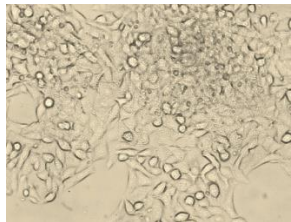
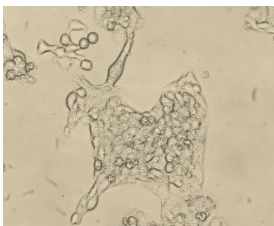
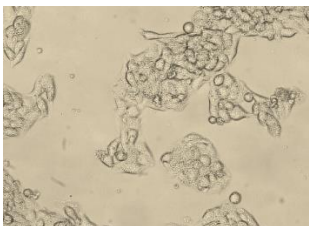
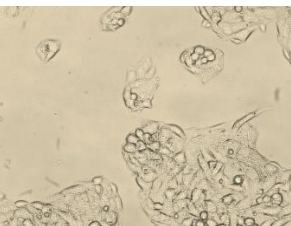
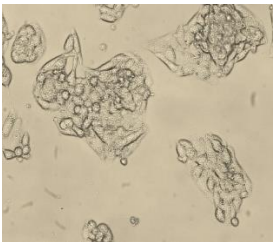
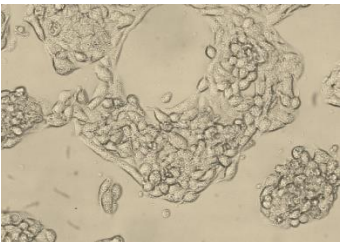
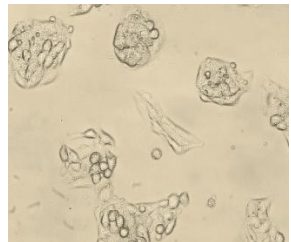
Sample concentration ($\mu\text{g/ml}$)	Percentage of inhibition (%)			
	Chloroform	Ethanol	Methanol	Ascorbic acid (Standard)
10	15.401 ± 0.008	26.767 ± 0.073	25.621 ± 0.027	27.724 ± 1.774
50	37.326 ± 0.018	36.468 ± 0.052	39.099 ± 0.008	57.474 ± 0.03
100	52.171 ± 0.032	55.32 ± 0.06	64.561 ± 0.008	61.079 ± 0.06
250	69.848 ± 0.078	80.592 ± 0.036	74.065 ± 0.15	67.967 ± 0.195
500	77.704 ± 0.039	89.976 ± 0.03	87.53 ± 0	71.166 ± 0.03
IC ₅₀	177.561	120.036	109.604	67.788

ANTICANCER ACTIVITY

Investigating the chemical components of *Connarus wightii* and testing its anticancer efficacy on hepatocellular cells Hep G2 were the goals of this work. Effects of *C. wightii* a extracts different solvent: methanol, ethanol, and chloroform treated with cancer cell lines HepG2 different concentrations plant compounds as the result Apoptotic cells shrink and have modifications to their plasma membranes as a result of caspase activation, which triggers the macrophage response (Hassan et al., 2014) . Methanol extracts of *C. wightii* were shown to be highly cytotoxic to human liver cancer (HepG2) cells, with low IC₅₀ values of 99.91, 158.12, and 47.42 $\mu\text{g/ml}$, respectively, in an anticancer examination conducted using the MTT assay. Sorafenib at a concentration of 30 μM served as the study's standard control. Table 4 shows that the methanol extract of *C. wightii* had substantial anti-cancer activity against HepG2, comparable to that of a conventional medication. HepG2 cells at various concentrations are shown in photomicrographic pictures in Figure-1. As well as being anti-inflammatory, many phytochemicals also function as regulators of the cell cycle and programmed cell death, which is how they cause cytotoxicity (Hanahan et al., 2000).

Concentration ($\mu\text{g/ml}$)	Percentage (%) of cell viability		
	Chloroform($\mu\text{g/ml}$)	Ethanol ($\mu\text{g/ml}$)	Methanol ($\mu\text{g/ml}$)
Control	100	100	100
Sorafenib(30 μm)	46.39 ± 1.41	46.39 ± 1.41	46.39 ± 1.41
12.5	78.99 ± 2.1	93.86 ± 1.7	78.99 ± 2.1
25	65.26 ± 2.07	88.59 ± 0.87	65.26 ± 2.07
50	50.81 ± 1.96	77.40 ± 2.3	50.81 ± 1.96
100	36.54 ± 1.25	67.21 ± 2.7	36.54 ± 1.25
200	12.25 ± 2.06	38.43 ± 0.34	12.25 ± 2.06
IC ₅₀	99.91	158.12	47.42

Chloroform Extract	Ethanol Extract	Methanol Extract

		
Control	Control	Control
		
Standard (30 μm)	Standard (30 μm)	Standard (30 μm)
		
12.5 μg/ml	12.5 μg/ml	12.5 μg/ml
		
25 μg/ml	25 μg/ml	25 μg/ml
		
50 μg/ml	50 μg/ml	50 μg/ml

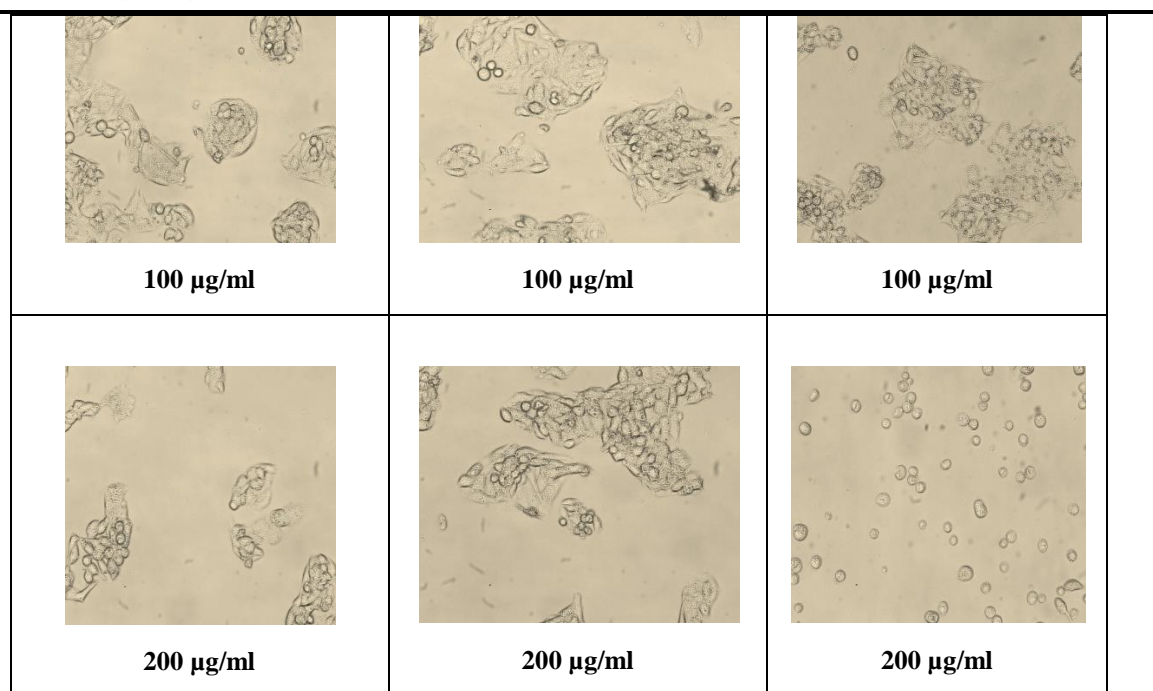


Figure-1. Photographic images show the effect of different solvent extract of *C. wightii* against HepG2cell line

Conclusions

To conclude, the present study explored the cytotoxic activity, photochemical analysis, anti-oxidant and of *Connarus wightii* plant. Three different solvents ethanol, methanol, and chloroform are used to extract the bioactive compounds from the leaves of *Connarus wightii*. Methanol extracts of *C. wightii* were shown to be highly cytotoxic to human liver cancer (HepG2) cells, with low IC₅₀ values of 99.91, 158.12, and 47.42 µg/ml, respectively, in an anticancer examination conducted using the MTT assay. Sorafenib at a concentration of 30 µM served as the study's standard control. The bioactive compounds present in the plant extract that have regulators the cell cycle and programmed cell death, as the result some bioactive compound present in this plant is usefully for therapeutic drugs for the treating cancer cells.

Acknowledgement

T.Maria Jenet (Reg:no:19121172272020) Acknowledges Annai Velankanni College, Tholayavattam- 629157 Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India, Providing the support for this research work

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