INVITRO ANTIOXIDANT AND CYTOTOXIC ACTIVITY OF PHYLLANTHUS EMBLICA BARK EXTRACT ON HEPG2 CELL LINE.

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Abstract:

Background: Hepatocellular carcinoma (HCC) is the most prevalent and deadly malignant tumor worldwide. *Phyllanthus emblica* (PE) has a long history of usage in traditional medicine for the treatment of cancer, liver illnesses, hyperglycemia, etc. In this study, we examined the anticancer activity of the bark of *Phyllanthus emblica* on HCC using the HepG2 cell line.

Aim: To analyze the cytotoxic activity of the bark of *Phyllanthus emblica* on the HepG2 cell line.

Methods: The MTT test was used to evaluate the cytotoxic potential of HepG2 cells after they had been subjected to varying doses of the extract. The antioxidant effect was measured using different assays like DPPH, $ABTS^{+}$, ferric reducing power, and the phosphomolybdenum assay. The extract was subjected to phytochemical analysis and phytochemical estimation like total phenol and flavonoid.

Results: It showed that the ethanolic extract of the bark of *Phyllanthus emblica* exhibited a potent cytotoxic effect on HepG2 with an IC_{50} of 12.89 µg/ ml, and the extract shown powerful radical scavenging activity with an IC_{50} value of 53.52 µg/ml, 41.07 µg/ml, 47.33 µg/ml, and 69.85 µg/ml using DPPH, $ABTS^{+}$, ferric reducing power and Phosphomolybdenum assay respectively. The bark extract had total phenolic content of GAE 145.89 µg/ml and flavonoid content of quercetin equivalent 80.57 µg/ml. In vitro study of the PE bark showed positive results against the HepG2 cell line.

Conclusion: As a result, the PE bark demonstrated antioxidant and anticancer activity. Hence, it has the potential to deliver promising therapeutic approaches against liver cancer.

Keywords: *Phyllanthus emblica* bark, hepatocellular carcinoma, HepG2 cell line, anti-cancer, anti-oxidant.

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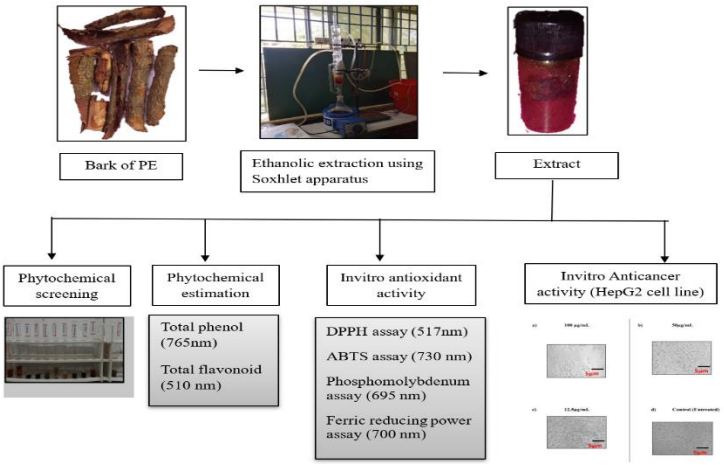
Graphical abstract:



Introduction:

The term "Cancer" refers to a vast variety of ailments that can harm any part of the body. Cancer can also be referred to as malignant tumors and neoplasms. Cancer is characterised by the accelerated proliferation of aberrant cells that surpass their typical growth boundaries, invade neighbouring regions of the body, and ultimately spread to other organs. The number one killer in the world is cancer. Globally, 9.5 million people died from cancer-related causes in 2018 out of 18.1 million new cases.[1,2] By the year 2040, it is anticipated that there will be 17 million deaths attributable to cancer and 30 million newly diagnosed cases of cancer each year. The third greatest cause of cancer-related fatalities globally is liver cancer. The sixth most prevalent cancer in the world is liver cancer.[3] The liver is a crucial organ in the body that performs a variety of functions, some of which include filtering the blood to remove wastes and foreign particles, controlling the levels of glucose in the blood, and producing nutrients that are necessary for survival.

Hepatocellular carcinoma (HCC), which affects adults, is the most typical form of primary liver cancer. The prevalence of hepatocellular carcinoma is two to four times higher in male than in female. Moreover, it is most prevalent in individuals who are between 40 & 65 years old.[4] Hepatocellular carcinoma comes from a combination of things, and the illness is very complicated. Hepatocarcinoma is most often brought on by a combination of factors, including the viruses that cause hepatitis B and C, AFLD, hyperglycemia, being overweight, and NAFLD.[5] Liver cancer may be impacted by several uncommon medical and hereditary factors, including smoking, food toxins, including aflatoxins, and numerous environmental chemicals.[4] Some metabolic conditions, like Wilson's disease, hepatic porphyria, and hereditary hemochromatosis, are linked to a greater risk of getting hepatocellular carcinoma.[6] Because of the enhanced inflammation and liver damage caused by these genetic illnesses, it is recognized that they encourage hepatocarcinogenesis.[7,8] According to the study, having both HBV and HCV infections increases the risk of developing HCC by 1.7%, and having both infections combined with alcohol use raises the risk by 4.2%.[9] As a result of the influence of hormones like estrogen, women are more likely than males to develop liver lesions and cirrhosis as a result of drinking alcohol. Three widely established pathways for ethanol-induced liver damage are hepatocyte fat accumulation, CYP2E1 activation, and oxidative stress-mediated hepatocyte destruction. Due to these alterations, hepatocytes experience inflammation and apoptosis.[10] According to certain research, NAFLD is still another risk factor for the development of HCC. 90% of those with obesity and about 70% of those with diabetes mellitus have NAFLD. Thus, NAFLD may increase the chance of developing HCC.[11]

Patients with HCC can choose from a wide range of treatments, such as hepatectomy, hepatic transplantation, drugs, percutaneous radio frequency ablation of liver tumors, and radiation. These treatments can be given systemically or through a blood vessel. If detected early enough, HCC is curable with surgical removal or liver transplantation. Early-stage HCC patients without cirrhosis should opt for surgical resection as their preferred treatment. Unfortunately, only 15% of HCC patients are suitable for curative therapy because the majority have severe illnesses and underlying liver damage. Also, the average time they live is less than a year, and their outlook is usually not good.[4,6] Patients with hepatocellular carcinoma are still hard to treat with palliative care because HCC is very resistant to systemic medicines.

Numerous molecular substances obtained from plants are effective against HCC. Herbal substances may have an impact on all stages of HCC, including initiation, promotion, and progression.[12] Due to their accessibility, affordability, and lack of unwanted side effects, plant-based therapies are now being investigated in cancer research. Certain plant compounds do not have an impact on healthy cells, but they are known to cause cancerous cells to undergo apoptosis.[13] The analysis of a certain medicinal plant from several angles may be the missing piece in the puzzle that unlocks the door to illness prevention and treatment.

In this study, we have analyzed the anticancer activity of the bark of *Phyllanthus emblica or Emblica officinalis* against hepatocellular carcinoma using HepG2 cell line. The Indian gooseberry, also known as amla, is known by its botanical name, *Phyllanthus emblica*. *Phyllanthus emblica* or *Emblica officinalis* is an arid and semi-arid tree that may be found in Madhya Pradesh, the southern part of ancient India, and coastal areas of India. It belongs to the family Euphorbiaceae. Throughout tropical India, *Phyllanthus emblica* is a moderate- to large-sized, big broad-leaf tree. *Phyllanthus emblica* has ashen bark and scarlet wood that can grow in the wild or cultivation and reach heights of 6 to 20 meters. The bark of *Phyllanthus emblica* is thick, up to 12 mm, and lustrous, appearing to be either greyish brown or greyish green. In Ayurvedic medicine, *Phyllanthus emblica* has a long history of usage as a treatment for fever, cancer, liver illnesses, diarrhea, inflammation, and cutaneous infections. The bark of *Phyllanthus emblica* was found to exhibit hepatoprotective activity against alcohol-induced toxicity. The bark of *Phyllanthus emblica* is a great source of gallic acid, flavonoids, and tannins[10]. According to numerous studies, phenolic and tannin-rich plants have strong anticancer properties[14]. In vitro and in vivo studies have shown that *Phyllanthus emblica* extracts are very effective in suppressing the growth of a variety of cancer types.[15] HepG2 cell line is one of the human hepatocellular carcinoma models that are used most often in experiments done in a lab. Hence, the primary objective of this research is to find out if *Phyllanthus emblica* bark extract can stop the growth of hepatocellular carcinoma.

Materials And Methods:

Chemical reagent:

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) were obtained from Invitrogen, USA. HepG2 cells were purchased from the National Centre for Cell Science in Pune. Rose-well Park Memorial Institute (RPMI) medium, amphotericin B (1mg/ml), 10% fetal bovine serum, gentamycin (100µg/ml), and penicillin/streptomycin (250 U/ml) obtained from Sigma Chemicals, MO, USA. Acridine orange was purchased from Sigma in the United States of America. Phosphate buffer, dimethyl sulfoxide, trichloroacetic acid, ethanol, phosphomolybdenum reagent, potassium ferricyanide, potassium persulfate, Ascorbic acid, 1, 1- diphenyl 2-picrylhydrazyl, sodium nitrite, ferric chloride, sodium carbonate, aluminium chloride, sodium hydroxide (NaOH), gallic acid, Folin Ciocalteau reagent, and quercetin were obtained from Sigma, Aldrich.

Plant collection and authentication:

Phyllanthus emblica bark was collected from Gummidipoondi village, Tiruvallur district, Tamil Nadu, India. The sample was authenticated at the Institute of Natural Science Research (INSR) by a botanist in Tamil Nadu. The voucher sample reference number 0065 has been given to a specimen of *Phyllanthus emblica* bark.

Plant extraction:

The bark of *Phyllanthus emblica* was carefully washed with tap water. A fresh sample is shade dried for a month; after that the dried bark of *Phyllanthus emblica* is then powdered with the help of an electric blender. Ethanol is used for the extraction of the *Phyllanthus emblica* bark with the help of a Soxhlet apparatus. For extraction, 100 - 150 g of *Phyllanthus emblica* bark in 500 ml of ethanol solution was used.

Phytochemical analysis of an ethanolic extract of *Phyllanthus emblica* bark:

Several phytochemical analysis were done on the ethanolic extract to find out what chemical constituents were in it. Alkaloids, quinone, terpenoids, phenolic compounds, flavonoids, tannins, carbohydrates, glycosides, saponins, proteins, and steroids were some of the phytochemicals that were investigated in this research.

Phytochemical estimation of an ethanolic extract of *Phyllanthus emblica* bark:

Estimation of total phenolic content: The Folin-Ciocalteau colorimetric technique was utilized to figure out how much phenolic acid was in the sample as a whole. After adding 900 µl of ethanol to 100 µl of the extract from the 1 mg/ml solution, the mixture was stirred. After that, 1 ml of a Folin-Ciocalteau reagent that had been diluted by a factor

of 1:10 was added. In addition to this, 1 ml of a solution containing 20% sodium carbonate was also added. Gave it a good shake, and the sample was then allowed to incubate for half an hour at room temperature in complete darkness. At a wavelength of 765 nm, absorbance was measured. After that, the results were contrasted with a gallic acid standard curve. This standard curve was generated using a known concentration range of gallic acid standards that were produced in the same manner as the sample. The findings were reported as gallic acid equivalents (GAE in µg/ ml).

Estimation of total flavonoid content:

The aluminium chloride ($AlCl_3$) - sodium nitrite ($NaNO_2$) technique was used to determine the overall flavonoid content. After adding 500 µl of ethanol to 500 µl of the extract from the 1 mg/ml solution, the mixture was stirred. To this, 1 ml of a sodium nitrite solution containing 5% is added. After that, 1 ml of aluminium chloride solution with a concentration of 10% is added, and after shaking, the mixture is incubated at room temperature for 5 minutes and 1 ml of 1 M NaOH solution was added, 30 minutes were given for the mixture to incubate at room temperature. Absorbance was calculated based on observations made at a wavelength of 510 nanometers. After that, the results were contrasted with a quercetin standard curve. The findings were reported as quercetin equivalent (QE in µg/ml)

Determination of antioxidant activity:

The antioxidant activity of *Phyllanthus emblica* bark was measured using the $ABTS^+$ radical cation scavenging assay, the phosphomolybdenum assay, the DPPH radical scavenging assay, and the ferric reducing power assay.

DPPH radical scavenging assay:

DPPH (1, 1- diphenyl- 2-picrylhydrazyl) radical scavenging activity was utilised in order to evaluate the radical scavenging capacity of the ethanol extracts. The sample at different concentrations (ranging from 50 to 300 µg/ml) was combined with 1 milliliter of 0.1 mM DPPH solution that was dissolved in ethanol. The mixture was then given thirty minutes to incubate in the dark. As a control, one milliliter of ethanol that had been combined with one milliliter of DPPH solution. At 517 nm, the decline in absorbance was observed and quantified. The gold benchmark for comparison was ascorbic acid [i.e., the standard]. The following formula was used to determine the % of inhibition:

% of $DPPH^{\cdot}$ radical inhibition = [(Control – sample)/ control] X 100

$ABTS^+$ radical cation scavenging assay:

Following the approach, the antioxidant capacity of the sample was determined using the radical cation scavenging activity of ABTS. After reacting 2.45 mM potassium persulfate with 7 mM ABTS stock solution and the mixture was then left to sit for 12–16 hours at room temperature without light. A 5 mM phosphate-buffered saline solution with a pH of 7.4 was used to dilute the ABTS solution, and the absorption at 730 nm was measured. The absorbance was calculated 10 minutes after the addition of ethanol extracts ranging in concentration from 10 to 60 mcg/ml to one millilitre of diluted ABTS solution.

The following formula was utilized to determine the % of inhibition:

% of ABTS radical cation inhibition = [(Control – sample)/ control] X 100

Ferric reducing power assay:

An analysis known as the ferric reducing antioxidant power that involves the utilization of antioxidants in the role of reductants in a redox-linked colorimetric reaction. With 1 ml of 1% potassium ferricyanide and 1 ml of phosphate buffer (0.2 M, pH 6.6), various quantities of the extracts (20–120 mcg/ml) were combined. For 20 minutes, the combination was left to incubate at 50° C. 10% trichloroacetic acid in a volume of 1 ml was added to the mixture, followed by the addition of 1 ml of freshly made 0.1% ferric chloride. The absorbance was measured at 700 nm.

% = [(Absorbance (sample) – Absorbance (control)/Absorbance (sample)] x 100

Phosphomolybdenum assay:

Applying a reduction assay method that relies on the generation of green phosphomolybdenum complex, the antioxidant activity was assessed. Extracts at different concentrations ranging from 20 to 120 mcg/ml were mixed with 1 ml of the phosphomolybdenum reagent. The containers were sealed, and a water bath set at 95°C was used to incubate them for 30 minutes. The components were utilised after cooling to room temperature and at 695 nm in comparison to a blank, the mixture's absorbance was determined.

% of Reduction = [(Absorbance (sample) – Absorbance (control)/Absorbance (sample)] x 100

Anticancer activity of ethanolic extract of *Phyllanthus emblica* bark:

HepG2 cell culture:

Human hepatocellular carcinoma (HepG2) cell lines were grown in Rose-well Park Memorial Institute medium (RPMI) with 10% fetal bovine

serum, 1 mg/ml amphotericin B, 100 g/ml gentamycin and 250 U/ml penicillin/streptomycin. At 37°C, all cell cultures were kept in humidified 5% CO_2 environments. Before use, cells were brought to confluence for 24 hours.

Cytotoxicity study by MTT assay:
The MTT reduction assay, as explained in the previous section with a few minor adjustments, was utilized to determine the cell viability. The HepG2 cells were inoculated at a density of 5 x 10^3 cells per well in 96-well plates for 24 hours, in a volume of 200 µl of RPMI containing 10% FBS. After that, the culture supernatant was taken out, and RPMI was added along with ethanolic extract of *Phyllanthus emblica* bark in different concentrations (1.56-100 µg/ml) and allowed to incubate for 48 hours. Following treatment, the cells were first incubated at 37℃ with MTT reagent (10 µl, 5mg/ml) for four hours, and then they were incubated at room temperature with DMSO for one hour. At a wavelength of 595 nm, the plates were scanned in a spectrophotometer. The percentage of cell viability was calculated, and IC_{50} values were calculated using nonlinear curve fitting[16].
% Cell viability = (mean OD/ Control OD) X 100
% growth inhibition = 100 - % cell growth

Results:
Phytochemical analysis: Table 1 illustrates the phytochemical analysis of *Phyllanthus emblica* bark extract, which revealed the presence of alkaloids, phenolic compounds, terpenoids, tannins, carbohydrates, flavonoids, quinone, steroids, and glycosides.

Table 1: Phytochemical analysis of PE bark extract

S.No.	Parameter	Results
1.	Alkaloids	+
2.	Tannins	+
3.	Phenolic compounds	+
4.	Glycosides	+
5.	Terpenoids	+
6.	Carbohydrates	+
7.	Saponins	-
8.	Flavonoids	+
9.	Quinone	+
10.	Proteins	-
11.	Steroids	+

Phytochemical estimation:
Table 2 illustrates the quantitative phytochemical screening of *Phyllanthus emblica* bark extract, which revealed the presence of total phenol and total flavonoids. The bark extract had total phenolic content above 100 µg/ml of gallic acid equivalents (i.e., 145.89 µg/ml) and flavonoid content below 100 µg/ml of quercetin equivalent (i.e., 80.57 µg/ml).

Table 2: phytochemical estimation of total phenol and total flavonoid

Parameter	**OD at 765nm**	**GAE (µg/ml)**
Total phenol	0.281	145.89
Parameter	**OD at 510nm**	**QE (µg/ml)**
Total flavonoid	0.450	80.57

Invitro antioxidant activity:
Table 3 illustrates the extract exhibiting potent radical scavenging activity with IC_{50} of 53.52 µg/ml, 41.07 µg/ml, 47.33 µg/ml, and 69.85 µg/ml using the DPPH radical scavenging assay, the $ABTS^+$ radical cation scavenging assay, the ferric reducing power assay and the phosphomolybdenum assay respectively.

Table 3: antioxidant activity of the sample

S.no	Method	IC_{50}
01.	DPPH radical scavenging activity	**53.52**
02.	$ABTS^+$ Radical cation scavenging assay	**41.07**
03.	Ferric reducing power assay	**47.33**
04.	Phosphomolybdenum assay	**69.85**

Anticancer property exhibited by an ethanolic extract of PE bark:

Cell viability MTT assay: Invitro study of ethanolic extract of *Phyllanthus emblica* bark showed positive results against HepG2 cell line, % of cell viability (growth) are 13.29%, 26.69%, 37.34%, 51.55%, 64.49%, 74.00%, 87.40% and % of growth inhibition are 86.71%, 73.31%, 62.66%, 48.45%, 35.51%, 26.00%, 12.60% for 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.56 µg/ml concentrations respectively. The average % of growth inhibition is 49.32%. (Table 4)

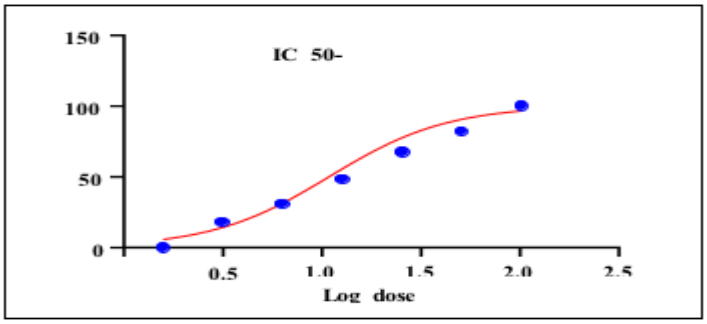
Table 4: Optical density readings for various concentrations of MTT assay

Conc µg/mL	OD1	OD2	OD3	Mean OD	% cell growth	%growth inhibition
100	0.12	0.1	0.13	0.116	13.29	86.71
50	0.21	0.25	0.24	0.233	26.69	73.31
25	0.32	0.34	0.32	0.326	37.34	62.66
12.5	0.42	0.45	0.48	0.450	51.55	48.45
6.25	0.56	0.54	0.59	0.563	64.49	35.51
3.125	0.62	0.63	0.69	0.646	74.00	26.00
1.56	0.75	0.76	0.78	0.763	87.40	12.60
Control	0.82	0.89	0.91	0.873	100	

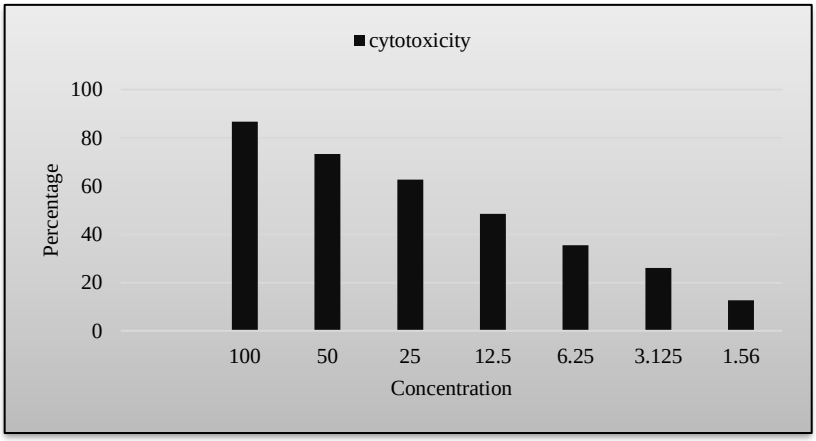
Cytotoxicity effect:

According to the findings, *Phyllanthus emblica* bark extract showed the maximum cytotoxic potency against HepG2 cells, with an IC_{50} of **12.89 µg/ml.** This suggested that the bark extract of *Phyllanthus emblica* was effective in inhibiting HCC cell proliferation. (Figures 1A,1B,1C, and 1D Represent the microscopic observations of HepG2 cells treated with ethanolic extract of *Phyllanthus emblica* bark at a concentration of 12.5 µg/mL, 50 µg/mL, 100 µg/mL, and control (untreated) respectively after 48 hr incubation) (Graph 1) [Graph 2 Cytotoxicity of ethanolic extract of *Phyllanthus emblica* bark against HepG2 cells after 48 hours of incubation (dose concentration range: 12.5-100 µg / ml)]

Graph 1: non-linear regression curve fit.



Graph 2: Cytotoxicity



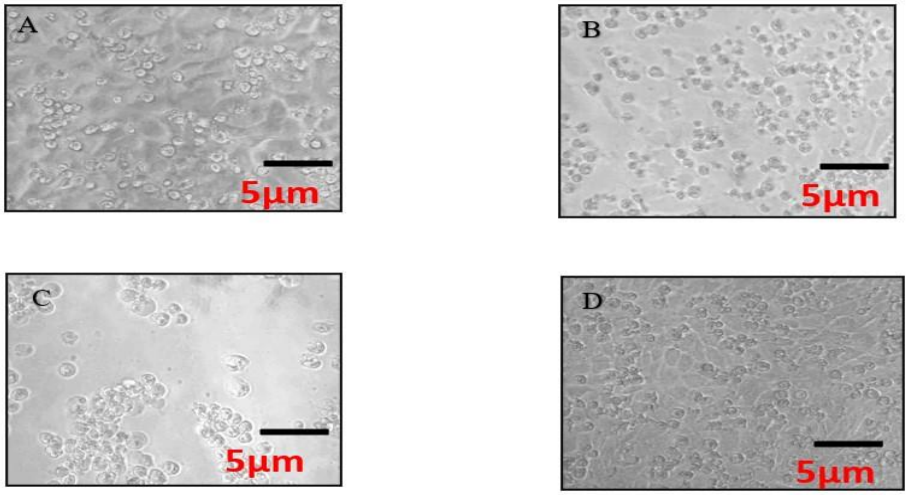


Figure 1 A, B, C, D: Microscopic observation of HepG2 cells treated with the sample.

Discussion:

The bark extract of *Phyllanthus emblica* exhibited a cytotoxic effect on HCC (HepG2 cells). In the current study, *Phyllanthus emblica* bark extract caused a significant decrease in the level of cellular viability depending upon the concentration range. Most likely, this extract's ability to limit cell growth by inducing cell death. In this way, the *Phyllanthus emblica* bark extract's inhibitory action supports the in vitro cytotoxicity. Preliminary phytochemical screening of *Phyllanthus emblica* bark extract as well as quantification of the *Phyllanthus emblica* bark extract verified that tannins, flavonoids, and phenols were present. In this study, the results of invitro antioxidant assays like the DPPH radical scavenging assay (IC_{50} value: 53.52 μg/ml), the ABTS assay (IC_{50} value: 41.07 μg/ml), the ferric reducing power assay (IC_{50} value:47.33 μg/ml) and the phosphomolybdenum assay (IC_{50} of 69.85 μg/ml) significantly demonstrated dose-dependent free radical scavenging ability. The findings of our investigation are consistent with those of other studies that have been done in the past.[10] It has been demonstrated that the *Phyllanthus emblica* extract possesses antitumor or cytotoxic effect in many different cancer cell lines, including KKU-452 cells[14], L929 cells[17], human leukemic (HK63) cells[18], MDA-MB-231 cells[19], Hela cells[19], MCF-7[20], and RMCCA1 [21]. In addition, the use of combination therapy in conjunction with standard chemotherapy not only improved the chemosensitivity of lung carcinoma and hepatocellular carcinoma cells[22] but also reduced the negative effects that are typically caused by chemotherapy.[23]

According to the findings of this research, the ethanolic bark extract of *Phyllanthus emblica* at concentrations of 12.5, 25, and 50 µg/ml exhibited an anti-proliferative effect. An anti-proliferative effect is one of the essential properties of chemotherapeutic agents. There is a possibility that *Phyllanthus emblica* might not have an immediate impact on cell growth; however, the chemical mechanism underlying this action still needs to be investigated. The level of antiproliferative activity was measured using the IC_{50}, where a lower value for the IC_{50} indicated a higher level of antiproliferative activity; consequently, this study reveals an IC_{50} value of 12.89 µg/ml for ethanolic extract of *Phyllanthus emblica* bark. An IC_{50} value of 52.2 µg/ml was found for the cytotoxic activity of a methanolic extract of the bark of *Phyllanthus emblica* by previous researchers.

According to one research, the *Phyllanthus emblica* extract at concentrations of 50 to 100 g/ml inhibited the growth of cancer cells by triggering caspase3/7 activity, DNA fragmentation, and the extrinsic apoptosis pathway, while concentrations of 50 and 25 µg/ml had an anti-invasive effect[19]. In different research, it was discovered that *Phyllanthus emblica* caused a greater tendency to encourage late death in cancer cells. Cells that had progressed to the later stages of apoptosis had lost the integrity of their plasma membranes and were stimulating various mechanisms of the inflammation pathway. Extrinsic and intrinsic pathways are both involved in the processes that cause cells to undergo programmed cell death (apoptosis). One potential mechanism for the bark of *Phyllanthus emblica* is the intrinsic pathway caused by reactive oxygen species-induced apoptosis.[14] In HepG2 cells, *C. speciosum, C. orientalis, G. deltonii, A. villosum, A. tatarinowii, and P. kesiya* were found to potentially trigger late apoptosis, DNA alkylation, and as a

result, DNA damage.[24] Apoptosis progresses to its final stage when DNA fragmentation takes place.[25] Activation of p53, and caspase-3 protease apoptotic cascades, were all implicated in the antiproliferative effects of *Phyllanthus emblica* extract.[21] In addition, it has been demonstrated that *Phyllanthus emblica* extract reduces thrombosis.[26] The phytochemical components found in the bark extract of *Phyllanthus emblica*, are essential for a chemotherapeutic effect targeting cell death in cancer cells. Based on these findings, it seems likely that the flavonoids and phenolic acids in the bark extract of *Phyllanthus emblica* are responsible for the antiproliferative effects. There is evidence that flavonoids and phenolic acids can disrupt the functioning of multiple signaling pathways involved in the carcinogenic process. The potential molecular targets include cell cycle halt induction and interference with some survival signaling pathways, including $STAT_3$ and NFκB.[27] Gallic acid has been demonstrated to have cytotoxic effects against cancer cells without affecting healthy cells; furthermore, it inhibits cell invasion, cell proliferation, and angiogenesis. These benefits come eventhough that it does not affect the normal cells.[28] Tannins are known to contain powerful anticancer activity, according to several studies. It was demonstrated that the *Phyllanthus emblica* bark extract was able to suppress the development of cholangiocarcinoma cells by inducing Reactive Oxygen Species in the cells.[6] After being exposed for 48 hours, the ethanolic extract of *Phyllanthus emblica* bark caused a decline in the viability of HepG2 cells. This decline was correlated with an enhancement in extracts concentration (figure). The cytotoxic impact was found to increase along with the concentration of the sample being tested. According to the findings of this research, the anticancer activity of *Phyllanthus emblica* was significantly higher in HepG2 cells. More research needs to be done to determine the mechanism underlying the anti-proliferative activity and molecular targets of action of *Phyllanthus emblica* bark against hepatocellular carcinoma need to be evaluated.

Conclusion:

The research concluded that the existence of effective chemical compounds in the ethanolic bark extract of *Phyllanthus emblica* is responsible for the anticancer activity that was observed when the HepG2 cell line was used to model hepatocellular carcinoma. The presence of phytoconstituents is proved with the help of phytochemical analysis and phytochemical estimation of total phenol and total flavonoid, which are believed to have the cancer-fighting abilities of the bark of *Phyllanthus emblica*. Cell viability MTT assay also shows significant growth inhibition. Additional research is required to decipher the mechanisms of anti-cancer activity and isolate active compounds from the bark of *Phyllanthus emblica* that have been shown to have strong cytotoxic effects. The *Phyllanthus emblica* bark product might be useful in the ongoing research and development of complementary and alternative cancer treatments for HCC patients.

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Conflict Of Interest:

none

Reference:

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