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Research Article

ANTICANCER EFFEECT OF THE EXTRACTS FROM *PHYLLANTHUS MADRESPATENSIS* AND *BREYNIA –VITIS- IDAEA* AGAINST LIVER CARCINOMA CELL LINES (HEPG2)

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A B S T R A C T

Cancer is one of the major health issues among the population all over the world, resulting in millions of diagnosis every year and increasing deaths resulting from this dreadful disease. Plants are a source of phytochemical compounds and secondary metabolites that play a major role in their medicinal properties. In this study, the active compounds from *Phyllanthus madrespatensis L.* and *Breynia vitis- idaea* (Burm.f.) C.E.C.Fisch. were evaluated in-vitro for their anticancer action against human liver cancer Hep G2 cell lines by MTT assay. The soxhlet extraction method prepared methanol extracts of *P. madrapatensis L.* and *B. vitis-idaea* (Burm.f.) C.E.C. Fisch. proved in-vitro cytotoxicity against human liver cancer cell lines. The methanolic extract of *P. madrapatensis L.* showed highest anticancer activity with 10µl (13.4 %), 20 µl (39%), 30 µl (41%) compared with the methanolic extract of the *B.-vitis-idaea* (Burm.f.) C.E.C.Fisch. The methanolic extract of the plant extract against Liver cell line could yield 80% growth of inhibition and were discussed.

INTRODUCTION

Cancer is one of the most dreaded diseases of the 20th century and spreading further with continuance and increasing incidence in 21st century. In the United States, as the leading cause of death, it accounts for 25% of all the deaths in humans presently. It is considered as an adversary of modernization and advanced pattern of socio-cultural life dominated by Western medicine. Multidisciplinary scientific investigations are making best efforts to combat this disease, but the sure-shot, perfect cure is yet to be brought into world medicine.

Cancer is the leading cause of mortality worldwide, and the failure of conventional chemotherapy to effect a major reduction in mortality indicates that new approaches are critically needed. The new and recent approach of chemotherapy serves as an attractive alternative to control malignancy (Kapadia *et al.,* 2000). In experimental cancer chemotherapy studies, attempts are made to identify agents which can exhibit any or a combination of the following characteristics: (i) prevent the initiation of tumors, (ii) delay or arrest the development of tumors, (iii) extend cancer latency periods, (iv)reduce cancer metastasis and mortality, and (v) prevent recurrence of secondary tumors. The major focus of research in chemotherapy for cancer in recent times includes the identification, characterization, and development of new and safe cancer chemopreventive agents (Kellof, 2000). Plants have played an important role as a source of effective anticancer agents, and it is significant that 60% of currently used anticancer agents are derived from natural sources, including plants, marine organisms, and microorganisms (Newman *et al.,* 2003; Cragg *et al.,* 2005). Plant-based medicine has definitely found a role in cancer treatment (chemotherapy), and the mechanism of interaction between many phytochemicals and cancer cells has been studied extensively (Kaufman *et al.,* 1999). In particular, there is growing interest in the pharmacological evaluation of various plants used in, Indian traditional system of medicine.

Plant-derived natural products such as flavonoids, terpenoids, and steroids, etc. have received considerable attention in recent years due to their diverse pharmacological properties, including antioxidant and antitumor activity (DeFeudis *et al.,* 2003; Takeoka and Dao, 2003). Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infection and degenerative diseases.

Phyllanthus madrespatensis is an erect or spreading sub shrub, growing to only 50 cm tall, well branched and hairless. It is also called as Madras Leaf flower as it is originated from the Madras region of India. The active constituents of *P. madrespatensis* are essential oil, Maderin, mucilage, β-sitosterol. The clear deep yellow oil can be extracted from the seeds of *P. madrespatnsis*. The seeds contain myristic,

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palmitic, stearic, oleic and linolenic acids and β-sitosterol. The deffated seed cake contains fibrous mucilage which can be hydrolysed to galactose, arabinose, rhamnose, and aldobionic acid.

Breynia vitis idaea (Burm.f.) is a perennial tree-like species of *Phyllanthaceae* (Euphorbiaceae s.l.), found from India east to Taiwan and Okinawa and south to Indonesia. The seeds are black and have a very hard seed coat. In this plant in roots contain β-sitosterol. Leaves contain triacontane, ceryl alcohol, lanosterol, pentatriacontanoic acid. Moreover, various *Phyllanthus* species has been reported to work against tumors and to have cytotoxic activities (Rajeshkumar *et al.,* 2002; Zhang *et al.,* 2004; Huang *et al.,* 2006; Tuchinda *et al.,* 2006).

Hepatotoxicity

The liver is an important organ that helps in the synthesis of specific biochemical substances that aids in digestion process, filtering of toxic metabolites (detoxification), glycogen storage regulation, and red blood cell decomposition (Francis, 1833). The liver can be damaged by certain toxic chemicals or drugs. These damages could lessen the efficiency of liver functions or completely disable the functions of the liver. Most of the liver injury happens due to the intake of drugs in overdoses. The term hepatotoxin represents all chemicals that cause liver damage. Drugs which cause hepatotoxicity have been removed from the market, and 50% of all acute liver failures are caused by drug-induced liver damage (Han *et al.,* 2013). The mechanism of hepatotoxicity is complex. Three-fourth of gastrointestinal tract blood goes to the liver through portal veins which brings foreign substance (drugs are recognized as foreign substance by the liver) in concentrated form. The excess drugs may cause mitochondrial damage which causes the release of free radicals and these free radicals, in turn, damage the hepatic cells and bile duct cells (Jaeschke *et al.,* 2002). The liver eliminates the drug using various enzymes but when overdosage of drug is taken, the liver cannot get rid of the massive amount of chemicals and finally, liver damage occurs. In many cases, if the drug intake is stopped, the liver functions come back to normal, but sometimes the damage is irreversible (Figure 1).

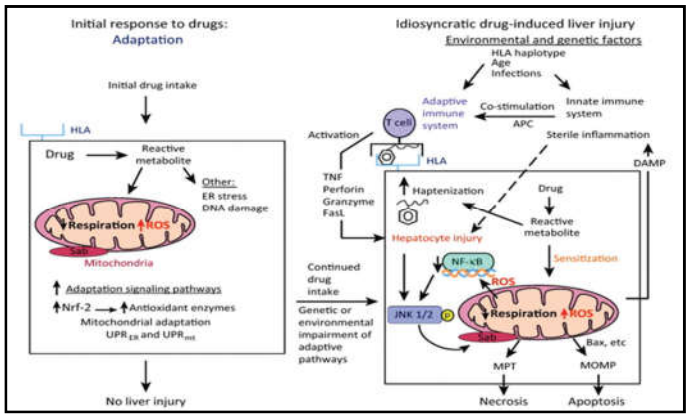

Figure 1 Mechanism of drug causing hepatotoxicity

The mechanism of hepatotoxicity is complex. The liver eliminates the drug using various enzymes but when overdosage of drug is taken, the liver cannot get rid of the massive amount of chemicals and finally, liver damage occurs (Han *et al.,* 2013).

Hepatoprotective Agents

Hepatoprotective agents, otherwise, called as antihepatotoxic agents are chemicals or drugs that lessen the hepatotoxins in the liver drastically, thus preventing the liver from further damage. These compounds, generally, prevent the liver from damage by chemicals. There are many drugs that provide antihepatotoxic activity, such as INH (isoniazid), rifampicin, pyrazinamide, phenylbutazone, allopurinol, erythromycin, and glibenclamide. However, the above drugs not only protect the liver from hepatotoxins but also cause many side effects such as stomach upset, fever, headache and vomiting. Therefore, the search for naturally occurring plant exhibiting antihepatotoxic activity with many bioactive compounds is undertaken by many scientists and research scholars, especially to avoid the side effects caused by current chemical drugs available in the market. Some of the plants were identified to provide antihepatotoxic activity. These plants include *Eclipta alba (*Franca *et al.,* 1995*)*, *Glycyrrhiza glabra* (Wan *et al.,* 2009)*, Boerhaavia diffusa* (Chandan *et al.,* 1991)*, Phyllanthus amarus* (Syamasundar *et al.,* 1985)*, Silybum marianum* (Madani *et al.,* 2008) and *Andrograhis paniculata* (Kapil *et al.,* 1993). However, the already reported plants were few and many traditional plants are still being studied extensively for antihepatotoxic activity. Hence, the present study was carried out to evaluate the anticancer activity of methanol extract of *Phyllanthus madrespatensis and Breynia vitis- idaea* against Hep G2 liver carcinoma cell lines.

Experimental

Collection and authentication of the plant

P.madraspatensis and *Breynia vitis-idaea (Euphorbi*aceae) were collected from the area of Thiruvarur Dt., and were identified and authenticated by the experts of Botanical Survey of India, Agricultural University at Coimbatore. A herbarium was deposited in the Department of Botany as BSI/SRC/5/23/2016/Tech.-1831 and BSI/SRC/5/23/2016/ Tech.-1830 in M.R.Govt Arts College Mannargudi.

Plant Material

The plant material was collected from Thiruvarur Dt. It was authenticated by Coimbatore. The plant was collected in the month of April 2015 and shade dried at room temperature.

Preparation of Plant extracts

The leaves were shade-dried and made into a coarse powder which was passed through a 40-mesh sieve to get a uniform particle size and then used for extraction. A weighed quantity (500 g) of the powder was then subjected to continuous hot extraction in Soxhlet apparatus with methanol, chloroform and Diethyl ether and the residual marc was collected. The extract was filtered through a cotton plug, followed by whatman filter paper (no.1). The extract was evaporated under reused pressure using a rotovac evaporator at a low temperature (40-60^{o}C) until all the solvent had been removed to give an extract sample with a yield of 18% w/w, 16 %w/w and 13% w/w in relation to the dried starting material. Preliminary Phytochemical analysis was carried out to identify presence of Phyto-constituents in the crude extract.

Anticancer activity

Cell line

Hep G2 Cell line were used for anticancer activity and for MTT assay method to identify the percentage of cell death. Cell line were initially procured from National centre for cell Science, Pune, India and has been maintained further in center for Bioscience and Nano science Research laboratory Echanari, Coimbatore, Tamil Nadu, India. The cells were maintained in RPMI 1640 supplied with 10% fetal Bovine serum, with glucose and sodium carbonate, 20 mg of ampicillin (2mg/ml). Cells were cultures for 3-4 days before the assay.

MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

Cell culture refers to culture derived from dissociated cells taken from the original tissue (primary cell culture). Cells are dispersed into a cell suspension which may then be cultured as a monolayer on a solid substrate, or as a suspension in the culture medium. Many animal cells can, with special care, be induced to grow outside of their organ or tissue of origin. Isolated cells, tissues or organs can be grown in plastic dishes when they are kept at defined temperatures using an incubator and supplemented with a medium containing cell nutrients and growth factors.

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 80-90% of humidity. Medium were replaced with serum free medium coating the sample in different concentration of 10-50 ml of sample, after slight mixing the plates were incubated for 4-24hrs at $37^{o}C$ in Co_2 incubator. The reaction mixture was then carefully taken out and formazan crystals were solubilized by adding 200 ml of DMSO to each well and mixed thoroughly. After 10 minutes the absorbance of purple color were read at 570 nm. After taking reading the % of cell death were calculated by following formula.

Percentage of cell death= control absorbance reading – absorbance of treated/ control absorbance reading X100.

Statistical analysis

All experimental results have been expressed as the mean ± standard deviation (SD). Statistical significance was calculated by ANOVA using GraphPad 7.0 software by Prism Inc. *P* value of lower than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

The liver is a sensitive organ and is more prone to toxic injuries than other organs. Several phytochemicals exhibit hepatoprotective effects in liver injury (Mittal *et al.,* 2012); however many are toxic, which limits their clinical use (Lagarto Parra *et al.,* 2001).

Subhashini *et al.* (2017) reported that the cell viability assay was further confirmed by cytotoxicity assay. The MTT assay reveals that the cell viability of Hep G2 cell lines decreases with increase in the concentration of plant extract. At 1.6 mg/ml (highest concentration tested), the cell toxicity was 64.6% while cell toxicity of positive control was 95.34%. The IC_{50} concentration was 1.38 mg/ml. The Hep G2 cell line death when exposed to plant extract shows that this plant exhibits antihepatotoxic property. A larger concentration of plant extract might completely kill the Hep G2 cells and since this plant has naturally occurring bioactive compounds, the side effects will also be very less.

In our present study, the MTT assay is based on the reduction of MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) by mitochodrial dehydrogenase to purple formazan crystals. *The different concentration of the compound isolated from the methanolic extract fraction of Phyllanthus madrepatensis and Breynia vitis idaea whole plant were subjected for MTT assay.* The cytotoxicity activity of methanolic extract of *Phyllanthus maderaspatensis* on liver cancer cell lines by MTT assay which was presented in table 1 and 2. A decrease in the cell count was observed with the increase in the concentration of the extract. There was a dose dependent increase in the cytotoxic activity. The *Phyllanthus madrespatensis extract* at low concentration (10µl) showed 13.4% cell inhibition and at high concentration (30µl) 41% cell inhibition the IC_{50} concentration was 1.86 and the same in expressed Table 1 and Figure 1. The *Breynia vitis idaea extract* at low concentration (10µl) showed 11% cell inhibition and at high concentration (30µl) 41% cell inhibition and the IC_{50} was 1.86 and the same in indicatedTable 2 and figure 2.

Table 1 Cytotoxicity activity using liver HEP G2 cell lines by MTT assay

S.No	*Phyllanthus maderaspatensis*	Conc.µl/ml	% cell inhibition	IC_{50} µl/ml
1		10 µl	13.4%	0.97
2	Methanolic	20 µl	39%	1.58
3	Extract	30 µl	41%	1.86

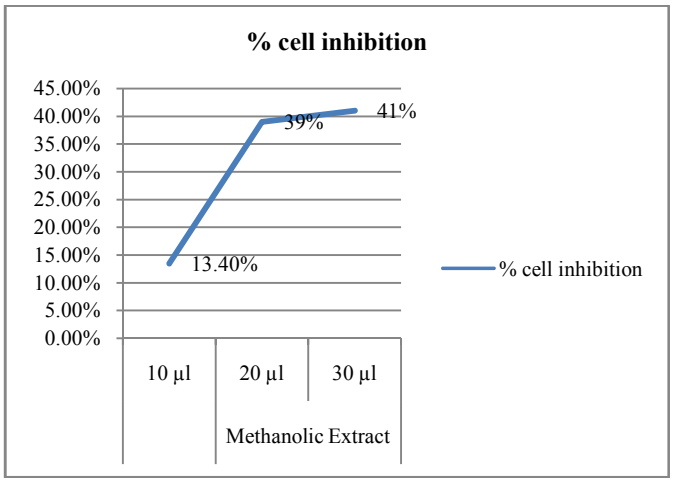

Figure 1 *Cytotoxicity activity using liver HEP G2 cell lines by MTT assay*

Table 2 Cytotoxicity activity using liver HEP G2 cell lines by MTT assay

S.No	*Breynia vitis idaea*	Conc.µl/ml	% cell inhibition	IC_{50} µl/ml
1		10 µl	11%	0.70
2	Methanolic	20 µl	39%	1.58
3	Extract	30 µl	41%	1.86

The 80% methanolic extract of *Phyllanthus madrespatensis* and *Breynia vitis idaea* exhibited significant anti cancer effect particulary for liver cancer (Figure-3). The anti cancer effect particularly against liver cancer on HEP G2 cell lines showed that the plant possesses anti cancer effect comparable to that of *Phyllanthus madrepatensis.* The anticancer activity studies have defined apoptosis as the pharmaco-dynamic endpoint for anti-cancer treatment (Kumar *et al.,* 2011).

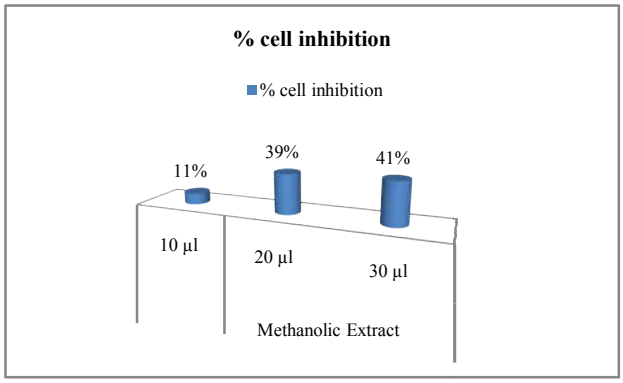

Figure 2 Cytotoxicity activity using liver HEP G2 cell lines by MTT assay+

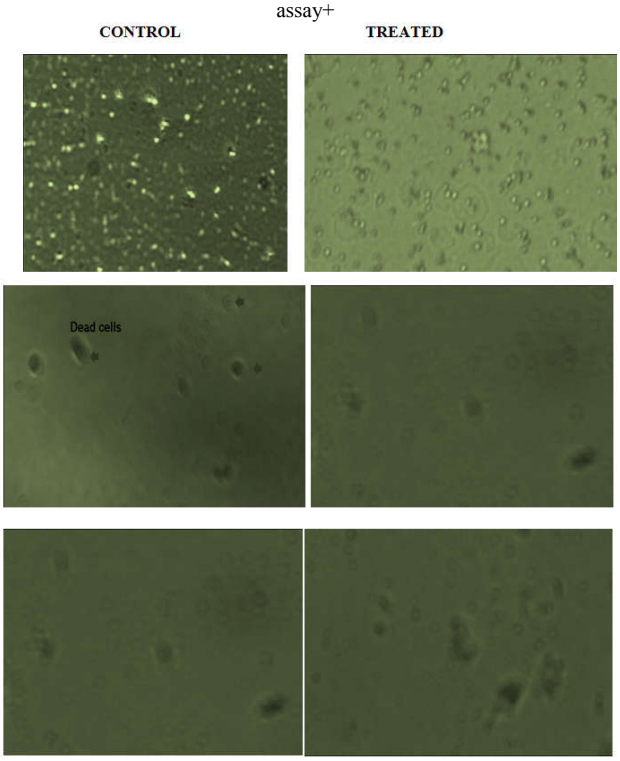

Figure 3 Cell Inhibition at various concentrations of 80% Methanolic extract after MTT treatment by using HEPG2 cell lines

Interestingly, the EW-L crude extract exhibited a higher selectivity than the melphalan and showed significant cytotoxicity and a high selectivity against HepG2 cells after 24 h exposure. Several researchers also reported in some medicinal plants (Chanda *et al.,* 2011; Nwaehujor and Udeh, 2011; Arijit *et al.,* 2011; Manosroi *et al.,* 2012; Ramamurthy *et al.,* 2014).

CONCLUSION

The results of the present study revealed that the MTT assay of the compound isolated from the methanolic extract of whole plant of *Phyllanthus madrepatensis* and *Breynia vitis idaea* shows that all concentration on having anticancer activity. The sample concentration of 10 µl/mg, 20 µl/mg and 30 µl/mg value against the human liver cancer HepG2 cell line respectively. Thus *Phyllanthus madrespatensis* to have the potential to act as a source of useful anticancer drugs and also to improve the health status due to the presence of compound that is vital for good health.

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