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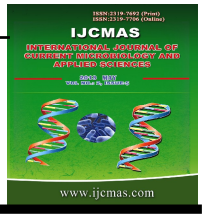

Original Research Article

Antioxidant and Antitumor activity of *Phyllanthus emblica* in colon cancer cell lines

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A B S T R A C T

The present study was designed to investigate the antioxidant and antitumor activity, of *Phyllanthus emblica* (fruit). Antioxidant potential of the edible plant was evaluated invitro by DPPH (1, 1 diphenyl – 2 picrylhydrazyl) scavenging assay and FRAP assay method. The radical scavenging activity of the extract was measured as decolourising activity followed by the trapping of the unpaired electron of DPPH. The percentage decrease of DPPH standard solution was recorded 71.75% for *Phyllanthus emblica* Phytochemical analysis revealed the presence of major phytocompounds like alkaloids, flavanoids, proteins, saponins and Tannins. The cytotoxic effect was determined against the cancer cells lines HT-29 using the MTT assay. In conclusion *Phyllanthus emblica* possess more potential cytotoxic activity against HT-29 cells lines .The result indicated that this plant extract could be an important dietary source with antioxidant & anticancer activities.

Keywords

Phyto-chemicals; antioxidant; cytotoxicity assay; *Phyllanthus emblica*

Introduction

Cancer is the abnormal growth of cells in our bodies that can lead to death. Cancer cells usually invade and destroy normal cells. These cells are born due to imbalance in the body and by correcting this imbalance , the cancer may be treated. Free radical, one of the major cause for the conversion of normal cell to cancerous cells,are generated as a consequences of a number of endogenous metabolic exposure to a plethora of exogenous chemicals (Rajkumar *et al.*, 2011). Antioxidant may mediate their effect by directly reacting with ROS, quenching them or chelating the catalytic metal ions (Sun *et al*., 2002). Antioxidants rich diets can reduce oxidative damage to DNA, thus preventing a critical step in the onset of carcinogenesis and the impact of antioxidants on mutagenesis and

carcinogenesis has been well established (Zhang *et al*.,2008;Meyskens *et al*., 2005). Chemoprevention, a novel approach for controlling cancer, involves the use of specific natural product or synthetic chemical agents to reverse, suppress or prevent premalignancy before the development of invasive cancer. Studies on a wide spectrum of plant secondary metabolites extractable as natural products from fruits, vegetables, teas, spices, and traditional medicinal herbs show that these plant natural products can act as potent anti-inflammatory, antioxidant or anticancer agents.

The recent advances in genomics and metabolomics have enabled biologists to better investigate the potential use of immunomodulatory natural products for treatment or control of various cancerous diseases. The cancer preventive or protective activities of the various immunomodulatory natural products lie in their effects on cellular defenses including detoxifying and antioxidant enzyme systems, and the induction of anti-inflammatory and antitumor or antimetastasis responses, often by targeting specific key transcription factors like nuclear factor kappa B (NF-kappaB), activator protein (AP-1), signal transducers and activators of transcription (STAT) and others (Arvindaram *et al*., 2 010).

There are numerous reports on cancer chemopreventive activity of dietary botanicals, includes cabbage, broccoli, garlic, onion, soybeans as well as medicinal plants. Several lead compounds, such as genistein (from soybeans), lycopene (from tomatoes) brassinin (from cruciferous vegetables) sulforaphane (from asparagus) are in preclinical or clinical trials for cancer chemoprevention since diet has an important role in the etiology of colon cancer ,dietary chemoprevention received attention for colon cancer prevention.

Phyllanthus emblica commonly known as gooseberry or aamla is a deciduous tree of phyllantaceae family. It has undergone preliminary research demonstrating in vitro antiviral,antimicrobial **(**Saeed *et al*., 2007) and anticancer activity (Ngam *et al*., 2010). *Phyllanthus emblica* has high level storage of polysaccharides,total protein and calcium(Suriyavathana and Subha, 2011) It is an oxidant with free radical scavenging properties due to the presence of high level of superoxide dismutage. It is rich in Tannin (gallicaid, elagic acid, phyllemblis acid, emblicol) (Anila *et al*., 2003). A wide range of phytochemical components including terpenoids, alkaloids, flavonoids, and tannins have been shown to posses' useful biological activities.

Plant anatomy

Kingdom	:	Plantae
Division	:	Flowering plant
Class	:	Magnoliopsida
Order	:	Malpighiales
Family	:	Phyllanthacae
Tribe	:	Phyllantheae
Subtribe	:	Fluegginae

Nutritive value

Amla is well known for its nutritional qualities. It is rich in polyphenols, minerals and is regarded as one of the richest source of vitamin C (200 - 900 mg per 100 g of edible portion) (Jain et al., 2000; Bharthakur et al., 1993; Gopalan et al., 1991). Major components of nutritional importance are reported in table 1.

Table. 1 Nutritional Value of fruit of *Phyllanthus emblica* (% or per 100g).

Chemical components	Percentage
Fruits: Moisture	81.2%
Protein	0.5%
Fat	0.1%
Mineral matter	0.7%
Fibre	3.4%
Carbohydrate	14.1%
Bulk elements Mg/100g	Net weight
Calcium	0.05%
Phosphorous	0.02
Iron	1.2 mg/100g
Vitamin C	600mg/100g
Nicotinic acid	0.2mg/100g

Materials and Methods

Sample collection

The samples of Phyllanthus emblica (fruit) were collected in Chennai, Tamilnadu (India). The fruit and leaves were dried.

Preparation of Extract

The plant material was shade dried with occasional shifting and the powered with a mechanical grinder, passing through sieve and stored in a tight container. Then 25gms of air dried powder of plant were continuously refluxed with ethanol at 45˚C for 3hrs using soxhlet apparatus. The mixtures were filtered. The filtrates were evaporated using vacuum rotary evaporator and air dried at 40°C. The stock solution of crude ethanolic extract were prepared by diluting the dried extracts with 0.25% dimethyl sulphoxide (DMSO) solution to obtain a final concentration of 100mg/ml.

Phytochemical Screening

The extracts was subjected to qualitative chemical investigation for the identification of different phyto constituents like steroids, tannins, flavanoids Alkaloids, Saponins, and Anthraquinones (Prashant Tiwari *et al*., 2011).

In- vitro Antioxidant Assay

DPPH Radical Assay

The ability of the extracts to scavenge DPPH free radicals was determined by the method (Gyamfi *et al*., 1999) with minor modification. The controls contained all the reaction reagents except the extract or positive control substance. Different concentration of the tested sample was placed in a cuvette and 0.5ml of 100mm methanolic solution of DPPH radical was added. Mixtures were vigorously shaken and left 30 min in the dark at ambient temperature. The absorbance was then measured at 517nm. Inhibition of DPPH radical was calculated as follows

DPPH radical % = (1- Absorbance of test / Absorbance of control)/ X 100

FRAP assay

FRAP reagents was freshly prepared by mixing 25ml acetate buffer(300mM,pH3.6),2.5ml 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ) solution(10mM TPTZ in 40mM/L HCL) and 2.5ml FeCl3(20mM) water solution. Each sample (150µl) (0.5mg/ml) of dissolved in methanol was added with 4.5ml of freshly prepared FRAP reagent & stirred and after 5 min, absorbance was measured at 593 nm using FRAP working solution as blank

(Szollosi and Szollosi Varga, 2002).The relative activity of the sample was compared with Ascorbic acid. FRAP value was calculated using the formula

FRAP value of sample (µM) = change in absorbance of sample from 0-4 mins / / change in Absorbance of standard from 0-4 min X FRAP value of std (100mM)

In -vitro cytotoxicity assay

Cell culture and experiment design

HT-29 cells are obtained from king's Institute, Chennai. The cells were grown and maintained in a humified incubator at 37*C under 5% Co2 atmosphere in MEM (Minimal Essential Media) medium supplemented with TPVG and 10% Fetal calf serum and (100 units/ml penicillin). For experiments, cells were plated in 48 well plates (at a density of$1x10^4$cells/ ml). After 25h incubation to allow cell attachment, the cells were treated with fresh medium containing different concentration of *Phyllanthus emblica* , dissolved in DMSO and incubated for 48hrs under same condition. Control groups received the same amount of DMSO.

Cytotoxicity Assay

The MTT colorimetric assay which is based on the reduction of MTT [3-(4,5-dimethyl]-2-thiazolyl)-2,5-diphenyl -2H tetrazolium bromide] by mitochondrial dehydrogenase to purple formazan product was used to assess the antiproliferative action of, *Phyllanthus emblica* extracts in human HT-29 cells. At the end of 48hrs incubation, the medium in each plate was added by 200µl of MTT solution and incubated for another 4h. The supernatant was then removed & replaced with 500µl of DMSO to dissolve the resulting MTT formazan crystals followed by mixing and measuring the absorbance using spectrophotometer at 590nm.

The cell viability % = 0.D of the sample/ 0 D of control / X 100 (Mossmann, 1983). To determination the % of cytotoxicity, graphs were plotted with the % of cytotoxities against their respective concentration.

Results and Discussion

Preliminary phyto chemical screening

In recent years attention has been focused on the antioxidant properties of plant derived dietary constituents of food (Gulein, 2006) The results of qualitative screening of phytochemical components in *Phyllanthus emblica*, were listed in Table.2. *Phyllanthus emblica* showed positive for all the test except steroids (Dhale and Mogle, 2011).

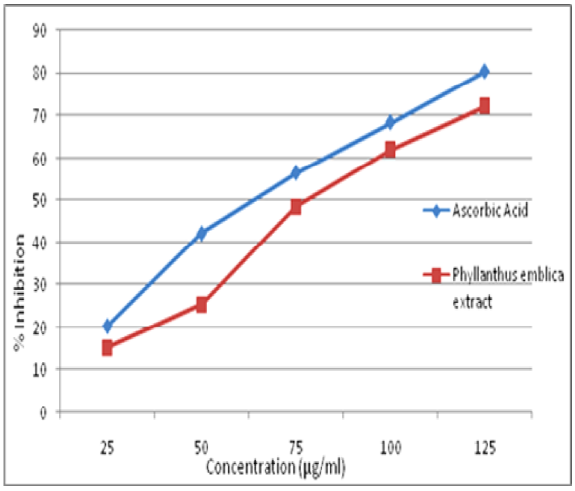
Table.2 Preliminary screening of phytochemical compounds in *Phyllanthus emblica*

Phytochemicals	***Phyllanthus emblica***
Tannins	+
Saponins	+
Flavonoids	+
Alkaloids	+
Proteins	+
Steroid	_
Anthraquinone	+

Determination of Free radical scavenging activity using DPPH method

DPPH test which is based on the ability of DPPH a stable free radical, to decolourize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517nm and also for a visible deep purple colour. when DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. *Phyllanthus emblica* extract showed high scavenging activity with 71.75% inhibition (Yadav *et al*., 2007) (Figure .1).

Figure.1 Free radical scavenging activity of different concentration of *Phyllanthus emblica* and ascorbic acid by DPPH radicals.

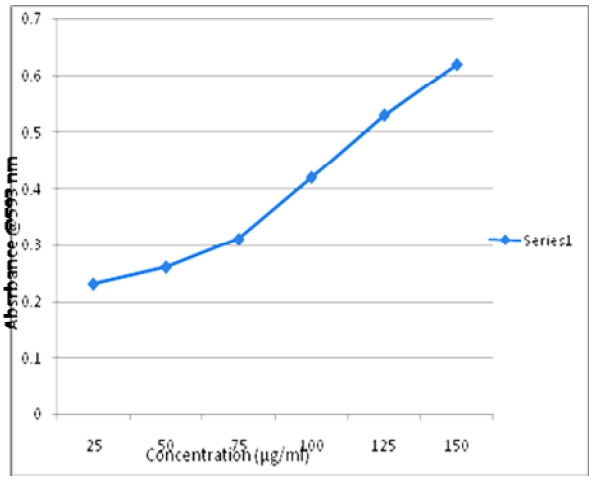


Phyllanthus emblica posses antioxidant and predominantly responsible for cytoprotective action in Non steriodal antiinflammatory drug induced ulcer.(Ananya chatterjee *et al*., 2011). .Additionally it has been determined that the antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as flavanoids,polyphenols,tannins, and phenolic terpenes (Rahman and Moon, 2007).

FRAP

Frap values for investigated extract are shown in Figure. 2 .The reducing power of the extract and the standard increased with the concentration. The reducing power of *Phyllanthus emblica* was more pronounced.The reducing power of a compound was related to its electron transfer ability and may, therefore serve as an indicator of its potential antioxidant activity (Sanchez –Moreno (2002).

Figure.2 Antioxidant activity measured by FRAP assay of *Phyllanthus emblica.*

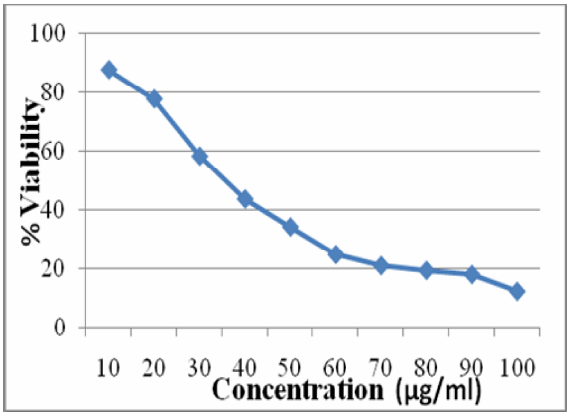


Anticancer activity

As a result of antioxidant activities *Phyllanthus emblica* were selected for further investigation on anticancer activities. The effect of ethanolic extracts on HT-29 cancer cell lines were evaluated

by MTT assay. Curves of percentage viability of treated cells were plotted against extracts concentration. The present study demonstrated the anticancer profile of *Phyllanthus emblica* effective against HT-29 cells The effect of *Phyllanthus emblica*,on Ht-29 cells is given figure.3. It can be found that the incubation of cancer cells with *Phyllanthus emblica*, reduced the viability of these cells and the dead cells were significantly increased with high extract concentration. The ethanolic extract of *Phyllanthus emblica* exhibited high cytotoxicity (88%) (Syam *et al* ., 2011) . *Phyllanthus emblica* showed the highest activity and this was observed in the earlier work with the HepG2 cells (Syam *et al* ., 2011). Even at very low concentration *Phyllanthus emblica* showed (20%) dead cells. In conclusion *Phyllanthus emblica* possessed strong antioxidant activity and anticancer activity.

Figure. 3 Antitumor activity of *Phyllanthus emblica* on HT-29 colon cancer cell lines



The results of the present study support the need of further studies to isolate potential anticancer drug with cancer cell-specific cytotoxicity. Additionally, the study supports the anticancer property of medicinal plants used in the traditional Indian medicine system and further evaluation of the selected medicinal plants for an effective anticancer drug with minimal side effects. Therefore supplementing a balanced diet with *Phyllanthus emblica* fruit may have beneficial effect.

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