

**INVITRO ANTI INFLAMMATORY AND ANTICANCER POTENTIAL
FROM THE ACETONE EXTRACT OF FRUIT OF *PHYLLANTHUS
ACIDUS. L***

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ABSTRACT

P. acidus, locally named as Arbaroi in Bangladesh and gooseberry or star gooseberry in India, is an edible small yellow berries fruit in the Phyllanthaceae family. To evaluate *invitro* anti inflammatory and anticancer potential from the acetone extract of fruit of *Phyllanthus acidus. L.* anti inflammatory activity was evaluated using membrane stabilizing activity method. The cytotoxic effect was determined against the cancer cell lines (A₅₄₉) using MTT assay. The extracts inhibited significant anti inflammatory and anticancer activities at dose dependent manner. Acetone extract showed superior activity which was comparable with standard drugs. At a concentration range of 2-10µg/mL it protects the human erythrocyte membrane against lysis

induced by hypertonic solution. At a concentration of 10 µg/mL the *P. acidus* fruit extract produced 74.1% inhibition of RBC haemolysis as compared with 80.01% produced by diclofenac sodium. Anticancer activity was found to be concentration dependent. At 1000 µg/mL concentration of *P.acidus* cell death was observed with least value of 14.2% respectively. The IC₅₀ value of fruit extract of *P. acidus* was found to be 61.5 µg/mL respectively.

KEYWORDS: *Phyllanthus acidus*, Anti inflammatory, anticancer.

INTRODUCTION

P. acidus, locally named as Arbaroi in Bangladesh and gooseberry or star gooseberry in India, is an edible small yellow berries fruit in the Phyllanthaceae family. Fruits are borne in loose clusters, are pale yellow or white, waxy, crisp and juicy, and very sour, found in

Bangladesh, South India, and Southeast Asian countries. The medicinal activities of *Phyllanthus* species are antipyretic, analgesic, anti-inflammatory, anti hepatotoxic and antiviral.^[1-4] Fruits of the two well-known species, *P. acidus* L. and *P. emblica* L. contain high contents of vitamin C and have been used for used for improving eyesight and memory and preventive action against Diabetes and relief of coughing.^[5] Another species of the family, *P. amarus* is an important herbal medicine due to its effective antiviral activities especially towards the hepatitis B virus.^[6-8]

Inflammation is the reaction of living tissues, to injury infection or irritation. Lysozymal enzymes released during inflammation produces a variety of disorders which leads to tissue injury by damaging the macromolecules and lipid peroxidation membranes which are assumed to be responsible for certain pathological conditions as heart attack, septic shock and rheumatoid arthritis etc.^[9] These inflammatory mediators come from plasma proteins or cells including mast cells, platelets, neutrophils, monocytes/ macrophages.^[10]

Cancer is the major public health problem, causing approximately 7 million deaths every year worldwide.^[11] More than 80% of cancer deaths are due to carcinomas such as lung, breast, prostate, colorectal, and pancreas cancers, which are currently the most lethal cancers.^[12] Lung cancer and colorectal cancers are responsible for the first and third most cancer related deaths in men and women. Breast cancer in women and prostate cancer in men rank second.^[13] Cancer is largely environmentally determined, being diet a major variable. Dietary patterns, foods, nutrients and other dietary constituents are closely associated with the risk for several types of cancer, and in this regard, it has been estimated that 35% of cancer deaths may be related to dietary factors. Recently, dietary polyphenols have received much attention for their anticancer properties.^[14;15] Many studies in different cell lines, animal models and human epidemiological trials suggest a protective role of dietary polyphenols against different types of cancers.^[16]

Chemoprevention is recognized as an important approach to control malignancy and recent studies have focused on the search for desirable chemopreventive agents. Natural products, particularly dietary substances, have played an important role in creating new chemopreventive agents.^[17]

According to Cragg and Newman (2000) over 50 % of the drugs in clinical trials for anticancer properties were isolated from natural sources or are related to them. Several

natural products of plant origin have potential value as chemotherapeutic agents. Some of the currently used anticancer agents derived from plants are podophyllotoxin, taxol, vincristine and camptothecin (Pezzuto, 1997). The areas of cancer and infectious diseases have a leading position in utilization of medicinal plants as a source of drug discovery. A great number of in vitro and in vivo studies have been developed to measure the efficiency of natural anticancer compounds either as pure compounds or as plant extracts.

MATERIALS AND METHODS

Plant Material

The plant material of fruit part of *P. acidus* were collected from in and around Chennai. It was identified using standard books. The fruit part were shade dried and crushed into fine powder with electric blender. The powdered sample was sealed in polythene bags and was stored in desiccators until further uses.

Preparation of acetone extract

Dried and powdered fruit part of *P. acidus* (500 g) were extracted using soxhlet with 100% acetone (1:5 W/V) for about 72 hours. The extracts was removed and it was concentrated to dryness in rotary vacuum evaporator below 50°C and stored until needed for the bioassays at -4 °C.

INVITRO ANTI-INFLAMMATORY ACTIVITY

Membrane stabilizing activity

Membrane stabilizing activity of the extract was assessed using hypotonic solution-induced rat erythrocyte haemolysis (Shinde *et al.*, 1999). The test sample consisted of stock erythrocyte (RBC) suspension (0.50 mL) mixed with 5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing the extract (0.25-2.0 mg/ml) or indomethacin (0.1 mg/mL). The control sample consisted of 0.5 mL of RBC mixed with hypotonic -buffered saline solution alone. The mixtures were incubated for 10 minutes at room temperature and centrifuged for 10 minutes at 3000 g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated using formulae.

$$\% \text{ Inhibition of haemolysis} = 100 \times (\text{OD}_1 - \text{OD}_2 / \text{OD}_1)$$

IN VITRO ANTICANCER ACTIVITY

Cell line and culture

Lung Cancer Cell line (A₅₄₉) was obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 µg/mL) in a humidified atmosphere of 50 µg/mL CO₂ at 37 °C.

Reagent

MEM was purchased from Hi Media Laboratories Fetal bovine serum (FBS) was purchased from Cistron laboratories Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

***In vitro* assay for Cytotoxicity activity (MTT assay).**

The Cytotoxicity of samples on A₅₄₉ was determined by the MTT assay (*Mosmann et al.*,1983). Cells (1×10^5 /well) were plated in 1mL of medium/well in 24- well plates (Costar Corning, Rochester,NY). After 48 hours of incubation, the cell reaches the confluence. Then, cells were incubated in the presence samples for 24 – 48 hours at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 100µL/well (5mg/mL) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)- 2,5-diphenyl--tetrazolium bromide cells (MTT) phosphate- buffered saline solution was added. After 4 hours incubation, Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC₅₀) was determined graphically. The absorbance at 570 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of A₅₄₉ was expressed as the % cell viability, using the following formula.

$$\% \text{ cell viability} = A_{570} \text{ of treated cells} / A_{570} \text{ of control cells} \times 100$$

RESULT

Anti inflammatory study was performed with acetone extract at a concentration range of 2 – 10µg/mL protects the human erythrocyte membrane against lysis induced by hypotonic solution. At a concentration of 10µg/mL the *P. acidus* fruit extract produced inhibition of RBC haemolysis as compared with diclofenac sodium. (Table: 1).

Table: 1. Anti inflammatory activity of acetone extracts of *P. acidus* (fruit)

SAMPLE	Concentration of acetone extracts ($\mu\text{g/mL}$)	Percentage of inhibition (%)	IC ₅₀
<i>P. acidus</i> (fruit)	2	40.99 \pm 0.71	3.66
	4	54.50 \pm 0.33	
	6	63.06 \pm 0.77	
	8	74.54 \pm 0.41	
	10	83.33 \pm 1.46	
Diclofenac sodium	2	58.66 \pm 0.58	7.89
	4	62.77 \pm 0.96	
	6	65.83 \pm 0.41	
	8	76.15 \pm 1.12	
	10	83.13 \pm 1.56	

Values are mean \pm standard deviation of triplicates.

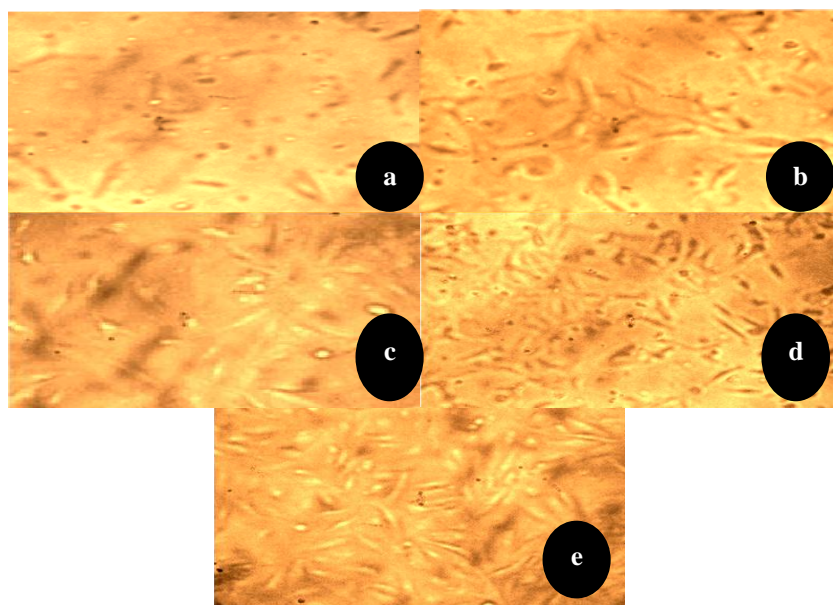
Anticancer activity

Anticancer potential of acetone extract was performed with DMSO control (used to dissolve extract). In order to confirm the anticancer activity of *P. acidus* fruit extract on A₅₄₉ cell line the cell viability percentage was observed by MTT assay. The cell survival percentage of the control cell have been shown in (Table:2) which is about 100%.

The viability of A₅₄₉ cells treated with acetone extract decreases in a concentration dependent manner, lower extract concentration exhibited stronger anticancer activity (Table:2; Figure1). Anticancer activity was found to be concentration dependent. At 1000 $\mu\text{g/mL}$ Concentration of cell death was attained with least value of 14.2% respectively. The IC₅₀ value from acetone extract of fruit of *P. acidus* was found to be 61.53 $\mu\text{g/mL}$ respectively.

Table: 2. Cell viability of *Phyllanthus acidus* (fruit) on lung cancer (A₅₄₉ cell line).

Concentration of acetone extracts ($\mu\text{g/mL}$)	Cell viability(%)
	<i>P. acidus</i>
	Fruit
1000	14.28 \pm 0.99
500	23.80 \pm 1.67
250	33.33 \pm 2.33
125	41.26 \pm 2.89
62.5	50.79 \pm 3.56
31.2	63.49 \pm 4.45
15.6	68.25 \pm 4.78
7.8	71.42 \pm 4.99
Cell control	100 \pm 0.00
IC ₅₀	61.53



a. 1000µg/mL. b. 125µg/mL., c. 62.5µg/mL. d. 31.2µg/mL. e. Control.

Figure : 1. Anticancer activity of acetone extract of fruit of *P. acidius*.

DISCUSSION

Inflammation involving the innate and adaptive immune systems is a normal response to infection. However, when allowed to continue unchecked, inflammation may result in autoimmune or autoinflammatory disorders, neurodegenerative disease, or cancer. A variety of safe and effective antiinflammatory agents are available, including aspirin and other nonsteroidal anti-inflammatories, with many more drugs under development. In particular, the new era of anti-inflammatory agents includes “biologicals” such as anticytokine therapies and small molecules that block the activity of kinases. Other anti-inflammatories currently in use or under development include statins, histone deacetylase inhibitors, PPAR agonists, and small RNAs (Charles, 2010). In Indian System of Medicine, the rhizome of *Cyperus rotundus* plant is recommended for use in different clinical conditions including fever and arthritis. The rhizomes are cooling, nerving tonic, and diuretic and traditionally used to treat diarrhoea, dysentery, leprosy, bronchitis and blood disorders. The rhizome is reported to posses analgesic, anti-inflammatory and antipyretic activity (Sharma *et al.*, 2010, Nagulendran *et al.*, 2007, Guldur, 2010).

According to Raja Chakraborty *et al.*, 2012 methanolic extracts were able to protect the erythrocytes against haemolysis in dose dependent manner and the protective effect of methanol was better than aspirin. The percentage inhibition of methanol extract from 100 – 200 µg/mL concentration were 78.19%, 86.09%, 53.58%, 58.49% against heat and hypotonic

solution induced haemolysis respectively. In the present study from the acetone extract of fruit of *P. acidus* produced 74.1% inhibition of RBC haemolysis as compared with 80.01% produced by diclofenac sodium. (Table:1)

Cancer development is a multistage process that involves a series of individual steps including initiation, promotion, progression, invasion and metastasis. Tumor initiation begins when DNA, in a cell or population of cells, is damaged by exposure to carcinogens, which are derived from three major sources: cigarette smoking, infection/inflammation, and nutrition/diet (Doll and Peto, 1981). If the DNA damage escapes repair, it can lead to genetic mutation. The resulting somatic mutation in a damaged cell can be reproduced during mitosis, which give rise to a clone of mutated cells. Tumor promotion is a selective clonal expansion of the initiated cells to form an actively proliferating multi-cellular premalignant tumor cell population.

Anticancer activity was found to be concentration dependent. In the present study, the Concentration of cell death was attained with the least value of 14.2% respectively. The IC₅₀ value from the acetone extract of fruit of *P. acidus* was found to be 61.53µg/mL respectively. According to Teijun *et al.*,2014 the cytotoxic effect of *P. emblica* were tested against triple negative breast cancer cells. The growth inhibition of MDA MB435 MDA MB468 MDA MB 231 and BT 20 cells exposed to 100µg/mL when compared to control ≤ 0.05 . The ethanolic extract of *P. emblica* exhibited highest cytotoxicity (88%) (Syam *et al.*,2011). The earlier work with HepG₂ liver cell line of *P. emblica* showed highest activity (Syam *et al.*,2011). Even at lower concentration (20%) dead cells were seen.

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