

## A COMPARATIVE EVALUATION OF ANTICANCER ACTIVITIES OF FLAVONOIDS ISOLATED FROM *MIMOSA PUDICA*, *ALOE VERA* AND *PHYLLANTHUS NIRURI* AGAINST HUMAN BREAST CARCINOMA CELL LINE (MCF-7) USING MTT ASSAY

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Received: 20 Jan 2014, Revised and Accepted: 01 Mar 2014

### ABSTRACT

**Objective:** The present study was designed to determine the comparative anticancer activities of flavonoids isolated from *Aloe vera*, *Mimosa pudica* and *Phyllanthus niruri* against human breast carcinoma cell line (MCF-7) using MTT assay.

**Methods:** MTT-based cytotoxicity activity study against human breast carcinoma cell line (MCF-7) was conducted to evaluate the potent activity of flavonoids isolated from *Aloe vera*, *Mimosa pudica* and *Phyllanthus niruri*. Thin Layer Chromatography (TLC) and Fourier Transform Infra-Red (FT-IR) spectra were recorded which confirmed the presence of flavonoids in the methanolic extracts.

**Results:** Flavonoids isolated from *Aloe vera*, *Mimosa pudica* and *Phyllanthus niruri* showed cytotoxicity activity against human breast carcinoma cell line (MCF-7) and the inhibitory concentration at 50% growth (IC<sub>50</sub>) was found to be, *Mimosa pudica* (IC<sub>50</sub>= 35.52±0.50µg/ml), *Aloe vera* (IC<sub>50</sub>= 54.97±0.36µg/ml) and *Phyllanthus niruri* (IC<sub>50</sub>= 84.88±0.87µg/ml).

**Conclusion:** The results indicated the cytotoxicity activity of all the three flavonoids isolated. Therefore, they can be effectively employed in anticancer treatment. Cytotoxic study suggested that flavonoid from *Mimosa pudica* has the maximum cytotoxic effect than flavonoid from *Aloe vera* and *Phyllanthus niruri* against MCF-7, Human breast cancer cell line (*Mimosa pudica* > *Aloe vera* > *Phyllanthus niruri*).

**Keywords:** *Aloe vera*, *Mimosa pudica*, *Phyllanthus niruri*, Flavonoids, MTT assay, Cytotoxicity.

### INTRODUCTION

Cancer is the major public health problem, causing approximately 7 million deaths every year worldwide [1]. 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 [2]. 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 [3]. 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 [4-6]. Many studies in different cell lines, animal models and human epidemiological trials suggest a protective role of dietary polyphenols against different types of cancers [1].

Flavonoids are a group of more than 4,000 polyphenolic compounds that occur naturally in foods of plant origin and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones [7]. The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. They have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor, antioxidant, antithrombotic, hypolipidemic and hypoglycemic activities [8-10].

*Aloe barbadensis miller* (*Aloe vera*), a member of the Liliaceae family, is a perennial succulent with turgid lace-shaped green leaves, and is referred to as the silent healer. *Aloe vera* extracts have been reported to have many biological activities such as hypoglycemic, hypolipidemic, antifungal, anticancer, antioxidant and immunoprotective properties [11-15].

*Mimosa pudica* (Family: Leguminosae) is a small or middle sizes tree about 1.5m in height with leaves are very sensitive, both pinnae and leaflets folding when touched. It is reported to contain alkaloid, glycoside, flavonoids and tannins. All parts of the plant are considered to possess medicinal properties. The plant has anti-

microbial, anti-convulsant, hyperglycemic, anti-oxidant, anti-venom, diuretic, anti-cancer, antidiabetic, anti-fertility and anti-histamine activities [16-18].

*Phyllanthus niruri* (*Euphorbiaceae*) originated in India and usually occurs as a winter weed throughout the hotter parts, contains over 600 species of shrubs, trees and annual or biennial herbs distributed throughout the tropical and subtropical areas. Whole plants have been used in traditional medicine for treatment of jaundice, asthma, hepatitis and malaria [19, 20]. It has a potent free radical scavenging activity and could scavenge superoxides, hydroxyl radicals and can inhibit lipid peroxides [21].

### MATERIALS AND METHODS

#### Cell Culture

The MCF-7 human breast cancer cell line was obtained from Rajiv Gandhi Centre for Biotechnology (RGCB), Department of Biotechnology (DBT), Government of India, Thiruvananthapuram, Kerala. The cells were grown in 60mm tissue culture dishes. It was maintained in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% Fetal Calf Serum, Penicillin G (100 U/ml), Streptomycin (100 µg/ml) and Gentamycin (50 µg/ml). Trypsin was employed for the successive passages.

When the cell culture were 80% confluent, the cells were subcultured at 1:2 splitting ratio. The cells were maintained at 37°C in a humidified 5% CO<sub>2</sub>/95% air atmosphere. All reagents were of tissue culture grade and were purchased from Merck, India Ltd.

#### Plant material

Leaves of the plant *Aloe vera* and the whole plants of *Mimosa pudica* and *Phyllanthus niruri* were collected from Kannur district of Kerala, India. After selection, plants were taxonomically identified by Dr. Sujanalal P, Scientist, Kerala Forest Research Institute (KFRI), Trissur, India.

#### Sample Preparation

The fresh leaves of the plant *Aloe vera* and the whole plants of *Mimosa pudica* and *Phyllanthus niruri* were thoroughly washed with

water. They were chopped into small pieces, dried in shade, grinded into powder form and stored in an air tight container.

### Extraction and Isolation procedure

The dried samples were Soxhlet extracted in 80% methanol (100ml/g dry weight) for 24hrs. The extracts were concentrated and reconcentrated in petroleum ether (40-60°C), ethyl ether and ethyl acetate. The ethyl acetate fractions which contained the highest amount of flavonoids were subjected to Column Chromatography over silica gel (60-120 mesh). Gradient elution was conducted initially with n-hexane and gradually enriched with benzene, chloroform, ethyl ether, acetone, ethanol, methanol and water successively in the order of increasing polarity [22]. Fractions were collected and combined on the basis of their TLC patterns. The fractions were then dried and analyzed for flavonoids.

### Test for Flavonoids

**Shinoda Test:** To the extract, added 5 ml of 95% ethanol and few drops of concentrated HCl. To this solution 0.5g of magnesium turnings were added. Observance of pink coloration indicated the presence of flavonoids [23].

A small quantity of the extract was heated with 10 ml of ethyl acetate in boiling water for 3 minutes and the mixture was filtered. The filtrate was then shaken with 1 ml of 1% aluminum chloride solution and observed for light yellow color. It indicated the presence of flavonoids. The yellow solution turns colorless on adding diluted NaOH and HCl, which confirmed the presence of flavonoids [24].

### Identification of flavonoids by TLC

TLC was performed for the identification of flavonoids. The concentrated extracts were spotted on the lower side of the TLC plate (20×20 cm) precoated with silica gel G. The diameter of each spot was about 5mm. Then TLC was run one dimensionally in the mobile phase solvent (ethyl acetate - methanol - water, 5:1:5, v/v/v) at room temperature. The plates were developed and visualizing under UV light [25, 26].

### FT-IR analysis

The column purified samples were mixed with 200mg KBr (FT-IR grade) and pressed into a pellet. The sample pellet was placed into the sample holder and FT-IR spectra were recorded (FT-IR Spectrometer-8400S Shimadzu).

### Cytotoxicity activity by MTT assay

The cytotoxic assay was carried out using MCF-7 human breast carcinoma cells maintained as monolayer cultures in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% Fetal Calf Serum, Penicillin G (100 U/ml), Streptomycin (100 µg/ml) and Gentamycin (50 µg/ml). Briefly  $1 \times 10^4$  cells  $\text{ml}^{-1}$  seeded in each well of a 24 well plate and incubated overnight at 37°C in a humidified 5%  $\text{CO}_2/95\%$  air atmosphere. The extracts were diluted in culture medium at different concentrations [27]. When the cells in the 24 well plate were 80% confluent, medium was removed from each well and extracts at different concentrations were added and kept for overnight incubation. Culture medium was replaced with fresh medium prior to the addition of 20µl of MTT solution (0.5mg  $\text{ml}^{-1}$ ). Treatment of living cells with MTT produced a dark blue formazan product, whereas no staining is observed in dead cells. The inhibitory concentration at 50% growth ( $\text{IC}_{50}$ ) was determined.

## RESULTS AND DISCUSSION

The methanol extracts of Aloe vera, Mimosa pudica and Phyllanthus niruri showed the presence of large number of compounds, fractionated with petroleum ether (40-60°C), ethyl ether and ethyl acetate. The ethyl acetate fractions which contained the highest amount of flavonoids were subjected to Column Chromatography. Gradient elution was conducted using solvents according to the increasing order of their polarity. Fractions were collected and tested for flavonoids and were found to be positive in acetone: ethanol (2:1 ratio). The fractions

were then dried and subjected to TLC which confirmed the presence of a single compound [Fig.1]. The methanolic extracts of Aloe vera, Mimosa pudica and Phyllanthus niruri confirmed the presence of flavonoids.

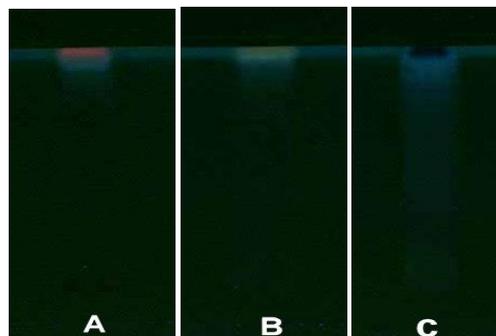
### Fourier Transform Infrared (FT-IR) analysis

FT-IR was used for identifying the functional groups and thereby confirming the isolates from *Mimosa pudica*, *Aloe vera* and *Phyllanthus niruri* were flavonoids. The functional groups present in the analyte will make vibrations of specific wave numbers. The spectral analysis is shown in Table: 1

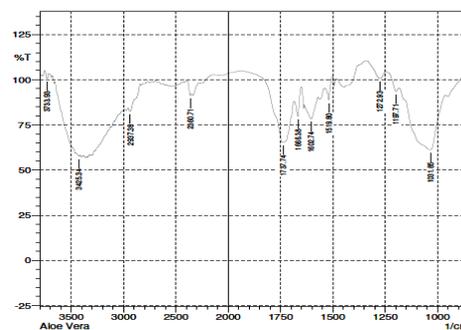
**Table 1: FTIR spectra analysis of Isolates from *Mimosa pudica*, *Aloe vera* and *Phyllanthus niruri***

Isolate from <i>Mimosa pudica</i> ( $\text{cm}^{-1}$ )	Isolate from <i>Aloe Vera</i> ( $\text{cm}^{-1}$ )	Isolate from <i>Phyllanthus Niruri</i> ( $\text{cm}^{-1}$ )	Indications
3419	3425	3425	O-H group stretching vibrations
2939	2937	2891	C-H stretching vibrations
1668	1666	1668	C=O aryl ketone stretching vibrations
1612	1602	1608	C---C aromatic ring stretching vibrations

The FT-IR fingerprinting provided the presence of OH stretched phenol, C=O aryl ketone and C---C aromatic ring in isolates from all the three plants; *Aloe vera*, *Mimosa pudica* and *Phyllanthus niruri*, which confirmed they were flavonoids.



**Fig. 1: It shows HPTLC profile of flavonoids isolated from *Mimosa Pudica* [A], *Aloe Vera* [B] and *Phyllanthus Niruri*; [C] Under UV 366nm.**



**Fig. 2: FTIR spectra of flavonoid isolated from *Aloe Vera* Cytotoxicity against MCF-7 Human breast cancer cell line**

The flavonoids isolated from the plants *Aloe vera*, *Mimosa pudica* and *Phyllanthus niruri* were tested for their *in vitro* cytotoxicity against MCF-7, Human breast cancer cell line. The inhibitory concentration at 50% growth ( $\text{IC}_{50}$ ) values of *Mimosa pudica*, *Aloe vera* and *Phyllanthus niruri* were found to be  $35.52 \pm 0.50$  µg/ml,  $54.97 \pm 0.36$  µg/ml and  $84.88 \pm 0.87$  µg/ml respectively.

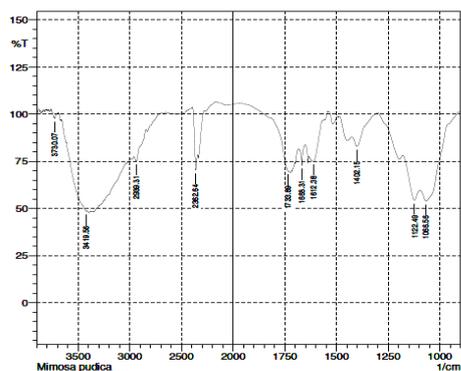


Fig. 3: FTIR spectra of flavonoid isolated from *Mimosa Pudica*

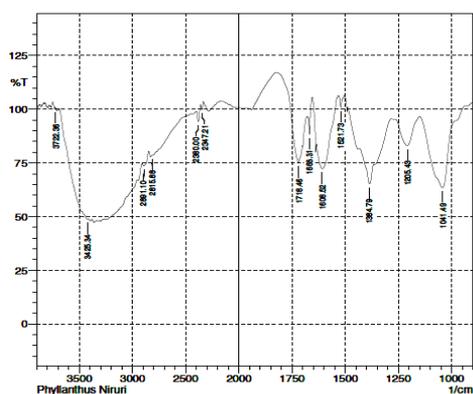


Fig. 4: FTIR spectra of flavonoid isolated from *Phyllanthus Niruri*

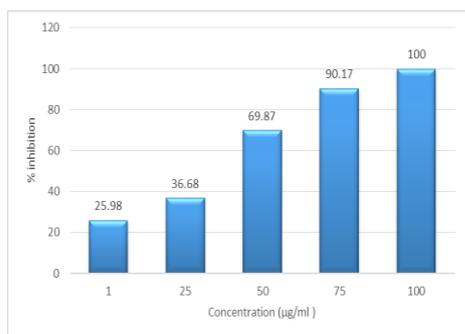


Fig. 5: Cytotoxicity activity of flavonoid isolated from *Mimosa Pudica* against MCF-7 Human Breast Carcinoma cell line

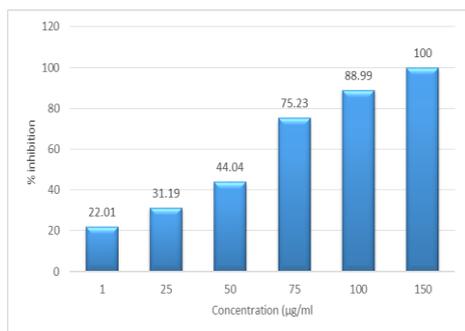


Fig. 6: Cytotoxicity activity of flavonoid isolated from *Aloe Vera* against MCF-7 Human Breast Carcinoma cell line

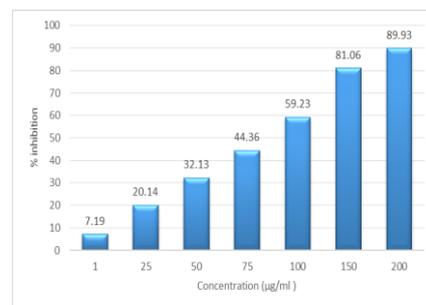


Fig. 7: Cytotoxicity activity of flavonoid isolated from *Phyllanthus Niruri* against MCF-7 Human Breast Carcinoma cell line

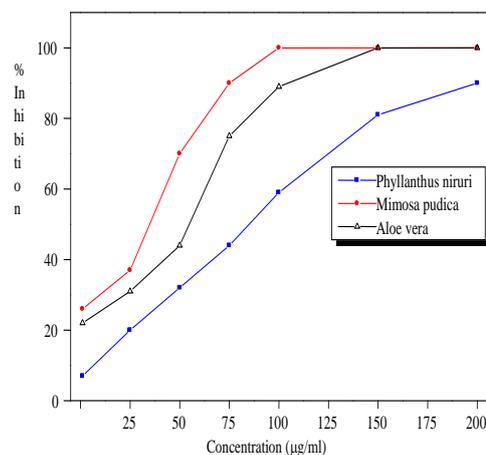


Fig. 8: Comparison of cytotoxic activity (% of inhibition) of isolated flavonoids from *Phyllanthus niruri*, *Mimosa pudica* and *Aloe Vera* at different concentrations (µg/ml)

## CONCLUSIONS

Bioactive flavonoids have been isolated successfully from *Mimosa pudica*, *Aloe vera* and *Phyllanthus niruri*, which were further confirmed as flavonoids as per the results obtained from TLC and FT-IR. All the isolated flavonoids showed potential anticancer activity. Cytotoxic study suggested that flavonoid from *Mimosa pudica* has the maximum cytotoxic effect than flavonoid from *Aloe vera* and *Phyllanthus niruri* against MCF-7, Human breast cancer cell line (*Mimosa pudica* > *Aloe vera* > *Phyllanthus niruri*). So the findings of this study could be considered as valuable information for the use of medicinal natural products in cancer treatment. Molecular level studies and investigations to characterize and elucidate the structure of the active principle behind the activity are under progress.

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