International Journal of Current Research in Medical Sciences

ISSN: 2454-5716

www.ijcrims.com

Volume: 1- Issue: 2- August- 2015

Research Article

In-vitro cytotoxicity and Phytochemical Activity in *Bryophyllum pinnatum*

Supriya Sharma , Shyam Kumar Shiv and Cheluvaraya Kannan Hindumathy*

*Department of Biotechnology,V.M.K.V. Engineering College, Vinayaka Missions University, Salem – 636 308.

*Corresponding author: *hindumathyck@rediffmail.com*

Abstract

BACKGROUND: Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals used for our various ailments). Progress over the centuries towards a better understanding of a plant derived medicine has depended on two factors that have gone hand in hand. One has been the development of increasingly strict criteria of proof that a medicine really does what it is claimed to do and the other has been the identification by chemical analysis of the active compound in the plant. **OBJECTIVE:** present study aimed on the *In vitro* anti-cancer properties of *Bryophyllum pinnatum* kurz extrac ts by MTT cell line by A 549 (Lung Cancer cell line) and MCF- 7 (Breast cancer cell line) **MATERIALS AND METHODS** The plant extracts were analyzed for presence of **Alkaloids, Flavonoids , Steroids, Terpenoids, Anthroquinones, Phenols, Saponins, Tannins** and isolated compound analysed for for the anticancer activitity. **RESULTS:** The phytochemical investigations of *Bryophyllum pinnatum* extracts (hexane, ethyl acetate and hydroalcohol) revealed differences in their phytoconstituents and anticancer activity, of cancer cell line A 549. The extract was tested for concentrations of 60.5, 125, 250, 500 and 1000µg/ml. Higher cytotoxicity is seen in 1000µg/ml with a value of 90.1% and CTC_{50} in µg/ml is 206. **CONCLOSION:** The highest yield was obtained in hydroalcohol and least yield was found in hexane. The phytochemical investigations of *Bryophyllum pinnatum* extracts (hydroalcohol, ethyl acetate and hexane) revealed differences in their phytoconstituents. While comparing the phytochemicals present in *Bryophyllum pinnatum* extracts, hydroalcohol extract found to be more active than other two extract sample. Total antioxidant activity of hydroalcohol extract of *Bryophyllum pinnatum*, showed that hydroalcohol extract possess high antioxidant activity.

Keywords: Plantextract,. Phytochemical and Anticancer.

Introduction

Plants have been used to treat or prevent illness since before recorded history. One of the oldest available literatures written around 2000 B.C. mentions the use of Cinnamon (*Cinnamomum verum*), Ginger (*Zingiber officinale*), Sandalwood (*Santalum album*) etc. used medical preparation [1].

Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals used for our various ailments [2]. Progress over the centuries towards a better understanding of a plant derived medicine has depended on two factors that have gone hand in hand. One has been the development of increasingly strict criteria of proof that a medicine really does what it is claimed to do and the other has been the identification by chemical analysis of the active compound in the plant [3]. (**Holiman, 1989**). The medicinal value of plants lies in some chemical

substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants [4].

Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical Constituents of plants would further be valuable in discovering the actual value of folkloric remedies [5]. Chemically constituents may be therapeutically active or inactive. The ones which are active are called active constituents and the inactive ones are called inert chemical constituents [6].**Iyengar, 1995**).

Several herbs and spices have been reported to exhibit antioxidant activity, including rosemary, sage, thyme, nutmeg, turmeric, white pepper, chili pepper, ginger, and several Chinese medicinal plants extracts [7] [8] [9] [10] [11] . The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, β-carotene, and α-tocopherol are known to possess antioxidant potential [12] [13] . A direct relationship between antioxidant activity and phenolic content of plant extracts has been reported [14] [15] Epidemiological studies have shown that the consumption of foods and beverages rich in phenolic content can reduce the risk of heart disease [16] .

In the present study the *In vitro* anti-cancer properties of *Bryophyllum pinnatum* kurz extracts by MTT cell line by A 549 (Lung Cancer cell line) and MCF- 7 (Breast cancer cell line) were studied.

Materials and Methods

Collection of Plant Materials

The fresh plant leaves of *Bryophyllum pinnatum* were collected randomly from the Yercaurd, Salem District, Tamil Nadu. Plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles in refrigerator.

Preparation of extract

Crude plant extract was prepared by Soxhlet extraction method. About 20gm of powdered plant material was uniformly packed into a thimble and extracted with 250ml of hexane, ethyl acetate and hydroalcohol extract separately. The process of extraction has to be continued for 24 hours or till the solvent in siphon tube of extractor become colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40ºC till all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC for the future use.

Phytochemical screening

The plant extracts were analyzed for presence of **Alkaloids, Flavonoids , Steroids, Terpenoids, Anthroquinones, Phenols, Saponins, Tannins**

Anticancer activity

Cell Lines and Culture Medium

A 549 (Lung Cancer cell line) and MCF- 7 (Breast cancer cell line) cell cultures were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in Dulbecco's modified Eagle's medium (DMEM). Medium was supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test Solutions

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serially two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of Cell Viability by MTT Assays

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using medium containing 10% FBS and were used for the determination of cell viability by MTT assays as described by [17] . respectively. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

$$\%\text{Growth inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

Results

The phytochemical investigations of *Bryophyllum pinnatum* extracts (hexane, ethyl acetate and hydroalcohol) revealed differences in their phytoconstituents (Table1). The hexane extract showed the presence of alkaloids, steroids, terpenoids, saponins, tannin and carbohydrate. In ethyl acetate extract alkaloids, flavonoids, steroids, phenol, carbohydrates and tannin are present. In the hydroalcoholic extract alkaloids, flavonoids, terpenoids, saponin, tannin, carbohydrates are present. While comparing the phytochemicals of *Bryophyllum pinnatum* extracts, hydroalcoholic extract is most active than other two extract

Table 1: Observation of phytochemical test:

S.No	Phytochemical Test	Observation	Inference
1	**Alkaloids**		
	Mayer's test	Formation of a yellow cream colour precipitate	Presence of alkaloids
	Wagner's test	Formation of a brown/ reddish brown colour precipitate	Presence of alkaloids
2	**Flavonoids**		
	Lead acetate test	Formation of a yellow colour precipitate	Presence of Flavonoids
	H_2SO_4 test	Formation of orange colour	Presence of Flavonoids
3	**Steroids**		
	Liebermann-Burchard test	Colour changed from violet to blue or green	Presence of steroids
4	**Terpenoids**		
	Salkowski	A reddish brown colour of the inner face	Presence of Terpenoids
5	**Arthoquinone**		
	Borntrager's test	Formation of a pink colour	Presence of arthoquinone
6	**Phenols**		
	Ferric chloride test	Formation of a bluish black colour	Presence of phenols
	Lead acetate test	Formation of a yellow colour precipitate	Presence of phenols

7	Saponin		
	Froth test	Formation of froth	Presence of saponins
8	**Tanin**		
	Ferric chloride test	Formation of a dark green colour	Presence of tannin
9	**Carbohydrates**		
	Fehling's test	Formation of a red colour precipitate	Presence of carbohydrate
10	**Oils & Resins**		
	Spot test	Appearance of transparency	Presence of oils & resins

Anticancer Activity

Leaf extract of *Bryophyllum pinnatum* was tested for the anticancer activity, of cancer cell line A 549. The extract was tested for concentrations of 60.5, 125, 250, 500 and 1000µg/ml. Higher cytotoxicity is seen in 1000µg/ml with a value of 90.1% and CTC_{50} in µg/ml is 206. (Fig-1 & Fig-3)

Fig 1 A 549 Cytotoxicity of (Lung Cancer cell line)

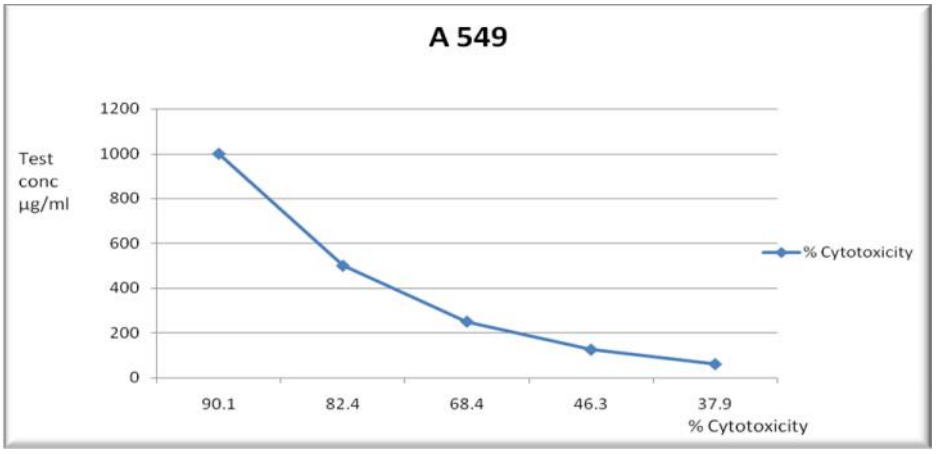
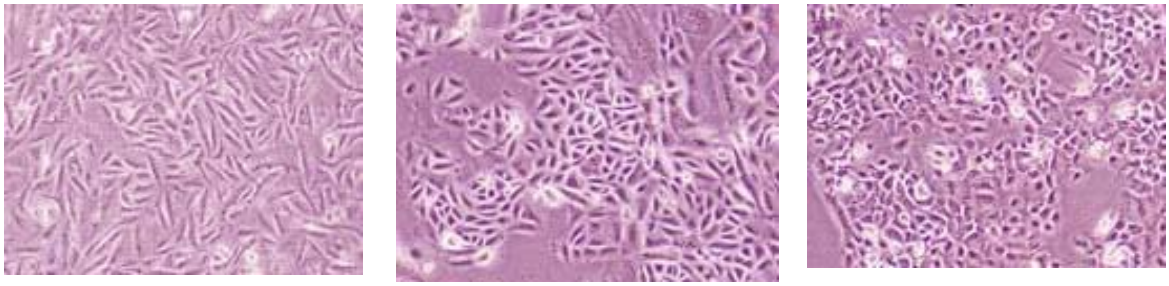
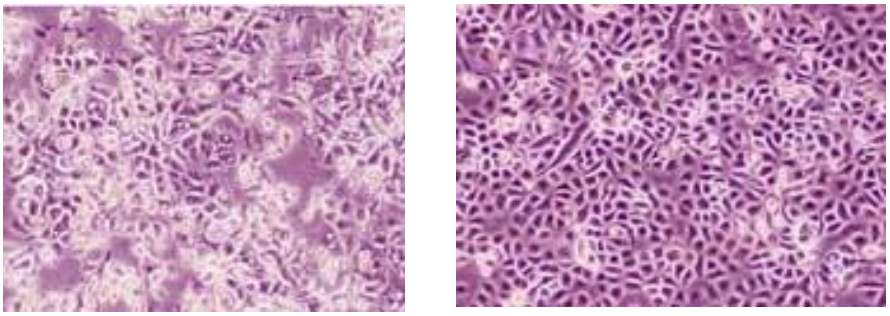


Fig 2: A 549 (Lung Cancer cell line)



1000 µg/ml **500 µg/ml** **250 µg/ml**



125 µg/m **60.5 µg/ml**

Fig 3: A 549 Cytotoxicity of (Lung Cancer cell line)

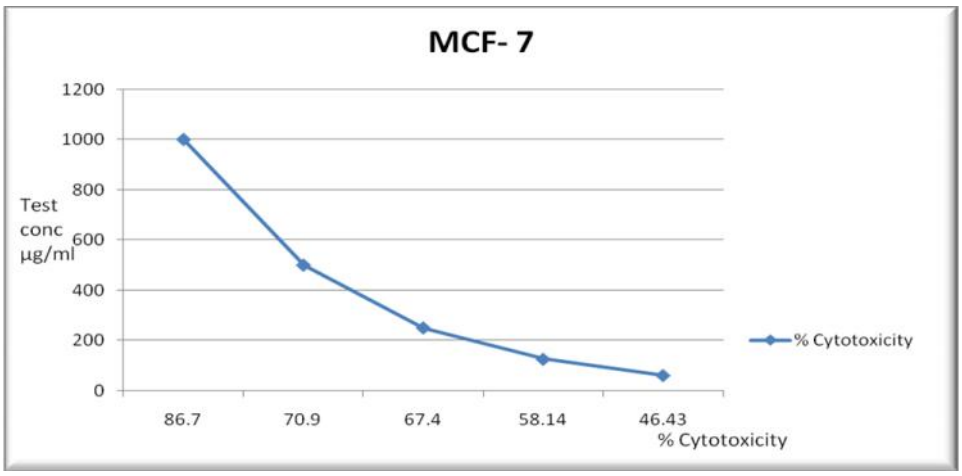
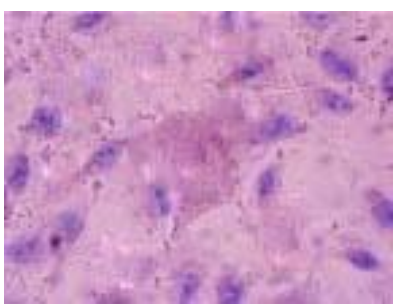
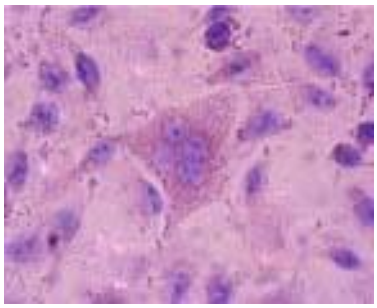


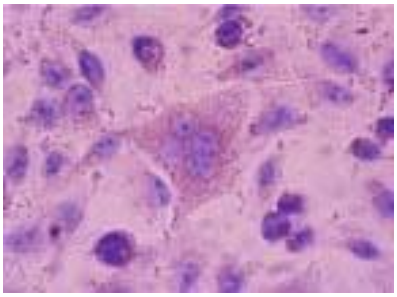
Fig 4: MCF- 7 (Breast cancer cell line)



1000 µg/ml

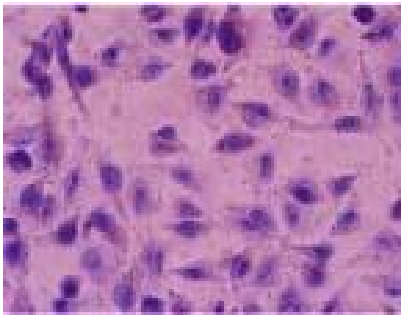


500 µg/ml



250 µg/ml

125µg/ml



60.5 µg/ml

Discussion

Many traditional plants remedies are known in folk medicine and used for treatment and some have been validated by scientific studies to actually exert biological action against wound healing or its complications.

This study therefore provided bases to the folkloric use of different plants as a remedy for cancer. It also justifies the folklore medicinal uses about the therapeutic values of these plants as curative agent and therefore, the purification and characterization of the phytochemicals that can be isolated from these plants will be useful as a chemotherapeutic agent [18]

A phytochemical analysis is very useful in the evaluation of some active biological compound of some medicinal plants. The qualitative and quantitative analyses of *Bryophyllum* pinnatum were carried out in each extract sample. Alkaloids, Saponins, Flavonoids, Tannins and Carbohydrate were revealed to be present in *Bryophyllum pinnatum* (table1).

The saponins content of *Bryophyllum pinnatum* justifies the use of the extract from this plant to stop bleeding and in treating of wounds [19].

Apart from saponins, other secondary metabolites constituent of *Bryophyllum pinnatum* found include alkaloids, tannins and Flavonoids. Flavonoids are potent water- soluble antioxidant and free radical scavenger, which prevent oxidative cell damage, have strong anti-cancer activity [20] . Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membrane. The alkaloids level of *Bryophyllum pinnatum* shows that the sample can be used as CMS stimulant and as powerful pain reliever [21].

The study therefore has provided some biochemical basis for the ethanomedical use of extracts from *Bryophyllum pinnatum* in treatment and prevention of infection and it can be potential source of useful drugs.

Further studies show the antimicrobial and antifungal activity of *Bryophyllum pinnatum* against the tested microorganisms. This suggests that the extract of this plant have broad spectrum in antimicrobial activity. Out of all the extract from leaves of *Bryophyllum pinnatum* leaves, the hexane extract was most active. 3 gram positive or gram negative bacterial strain and 3 fungal strains were used. Extract from the leaves of *Bryophyllum pinnatum* showed significant effect on tested microorganism, this justifies the traditional use of the juice obtained from the slightly heated fresh leaves of this plant against antimicrobial infection like skin infection, wound infection, abscess and gastrointestinal disorder [21] . (Highest antimicrobial activity is seen in the organism *S.boydii* in the Hexane extract. For the other two organisms the highest activity is seen in Hexane and the activities are higher than the control. The highest activity in antifungal is seen in the Hexane extract of organism *C.lunata* and the activity is higher than the control. In the organism *T.rubrum*, the highest activity is seen in the Hexane and for the organism *A.niger* the highest activity is seen in Ethyl acetate.

In living system, free radicals are constantly generated and they can cause extensive damage to tissues and biomolecules leading to various disease condition especially degenerative disease and extensive lysis. Many synthetic drugs protect against oxidative damage but they have adverse side effect [22] An alternative to the problem is to consume natural antioxidant from food supplements and traditional medicines have been isolated from different plant materials [23] Flavonoids are effective antioxidant mainly because they scavenge superoxide anions. The result suggests that the plant extract is a more potent scavenger of superoxide radical than the standard ascorbic acid. Hydroalcoholic extract of *Bryophyllum pinnatum* has antioxidant activity. Antioxidant activity of the *Bryophyllum pinnatum* was measured by reducing power assay and total antioxidant activity method. In both methods result is higher than the standard values.

In India highest motility due to cancer in men and women is because of lung and breast cancer. So in this study it was found that extract from *Bryophyllum pinnatum* has anticancer activity. *In vitro* anticancer activity was measured by MTT assay. It is a colorimetric assay for assessing cell viability.MTT assay reveled that the hydroalcohloic extract from *Bryophyllum pinnatum* is more effective against breast cancer as it reaches to CTC_{50} value at 75.4µg/ml in comparison to lung cancer whose CTC_{50} value reaches at 206µg/ml. This shows that it has potential to cure cancer and can be used for making drug for the treatment.(Fig 2& Fig 4)

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