

**EVALUATION OF ANTI-CANCER ACTIVITY AND PHYTOCONSTITUENTS OF
BIOPHYTUMSENSITIVUM LINN: AN INVITRO STUDY****Tharani M.*¹, Tamilselvan T.², Ponnudurai K.¹, Malaisamy N.¹, Vignesh Mirthick R.¹, Wafa Ahmed Saadeldin Badr¹ and Sabari Murthy V.¹**¹Department of Pharmacology, Cherraan's College of Pharmacy, Telungupalayam Pirivu, Coimbatore-641039, Tamilnadu, India.²Department of Pharmacy Practice, Nehru College of Pharmacy, Pambady- 680588, Kerala.***Corresponding Author: Tharani M.**

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ABSTRACT

Objective: In the current research phytochemical and invitro, anticancer activity was evaluated using petroleum ether extract and methanol extract of *Biophytumsensitivum Linn*. **Materials and Methods:** The whole plant was dried for fifteen days and grounded using mechanical blender. The extraction was carried out using the Soxhlet apparatus with 10 volumes of 75% Methanol and another extraction with Petroleum Ether (10 volumes of 75% solution). The extract was evaporated and dried. Preliminary phytochemical screening and FTIR techniques were performed to identify the basic components. Using DLA and EAC cell lines the anti-cancer activity was carried out. **Results:** The whole plant showed the presence of phenols and flavonoids at a higher rate than steroids, glycosides, and Ketones. Its presence was confirmed using FTIR reports. The cytotoxicity studies were highly significant in Methanol extract of *Biophytumsensitivum Linn* than Petroleum Ether extract. **Conclusion:** The effective anti-cancer activity was reported in the whole plant Methanolic extract of *Biophytumsensitivum Linn* as compared with petroleum ether extract. Further specific studies are required to find out the specific individual components

KEYWORDS: *Biophytumsensitivum Linn*, Phytochemical screening, DLA, EAC cell lines.**INTRODUCTION**

Tumors are maladies that include irregular multiplication of cells with the possibility to attack and spread to different parts of the body.^[1] All tumors show a significant change in the characteristics of cells to form a malignant tumor which includes cell growth and division suppresses proper signaling pathways, abnormal proliferation, and division in the absence of cell signals, depletion of apoptosis, construction of blood vessels (Angiogenesis promotion) and invade tissues and enhance metastasis.^[1-2]

This abnormal growth accounts for 90-95% cases due to gene mutation which resulted from environmental and lifestyle modification. 5-10% due to inherited genes. Environmental factors include lifestyle, economic and behavioral factors. Common factors are tobacco usage (25-30% cases), diet and obesity (30-35% cases), infection (15-20% cases) and radiation (10% cases), stress, lethargy, and pollution.^[3] The systemic symptoms include unintentional weight loss, fever, excessive fatigue, and skin discoloration. Hodgkin's disease, Hepatic and Kidney tumors, Blood cancers can cause persistent fever.^[4] Chemotherapy is a treatment that helps with one or more cytotoxicity, acts by killing cells that

divide rapidly.^[5] The effectiveness of chemotherapy is often limited by its toxicity to other tissues in the body.^[6] A variety of therapies using immunotherapy also emerged since 1997^[7], people preferred an alternative system of medicine due to low cost and lesser toxicity.

Most of the complementary and alternative medicine for cancer has not been tested using conventional techniques like clinical trials.^[8] Hence the present study was carried out to scientifically investigate in-vitro anti-cancer activity using *Biophytumsensitivum Linn* to promote an effective treatment regimen. It is widely used in traditional medicine which is a small annual herb belonging to the family oxalidaceae.^[9] Since the plant is used as folk medicine it can be used to screen invitro anticancer activity.^[10]



Fig. 1: *Biophytumsensitivum Linn(DC)* plant with flower.

MATERIALS AND METHODS

The Matured and healthy whole plant of *Biophytumsensitivum Linn* were collected from Pachilai Mooligai at Kolli hills and authenticated from the department of a botanical survey of India, Tamilnadu Agricultural University (TNAU) Southern Regional Centre, Coimbatore.

Methanol Extract

After collection, the whole plant was shade dried for fifteen days and ground using mechanical tissue blender. The air-dried whole plant (250 g) was powdered and extracted using the soxhlet apparatus overnight by maceration with 10 volumes of 75% methanol. The solvent was evaporated to dryness at (10 to 40°C) under reduced pressure using a rotary evaporator. The yield of the extract was 18%.

Petroleum Ether Extract

Biophytumsensitivum Linn was sequentially extracted. The air-dried whole plant (250 g) was powdered and extracted with using soxhlet apparatus overnight by maceration with 10 volumes of 75% petroleum ether. The solvent was evaporated to dryness at (10 to 40°C) under reduced pressure using a rotary evaporator. The yield of the extract was for 16%.

Tab. 1: Presence of Phytoconstituents of *Biophytumsensitivum Linn*.

S.No.	Test Parameter	Methanol Extract	Petroleum Ether Extract
1.	Phenols	+++	++
2.	Flavonoids	+++	++
3.	Steroids	++	+
4.	Glycosides	++	+
5.	Glucose	++	+
6.	Fatty acid	-	-
7.	Ketones	-	-

+++ Highly Present ++ Moderately Present + Mildly Present - Absent

FTIR Analysis of petroleum ether extract of *Biophytumsensitivum Linn*.

FTIR analysis was used to find out the phytoconstituents type using functional groups. Aromatic (C C Stretching) peak was observed at 1467.83 in Petroleum Ether Extract

Presence of Phenol, Flavanoids, Glycosides, Steroids, Fatty acids, Ketones and Glucose were screened by using the suitable chemical test like Ferric Chloride Test, Foam Test, Lieberman Burchard Reaction, Spot Test, Sodium Bisulphite Test, Meta Di Nitro Benzene Test and Benedicts Reagent Test respectively.

Invitro Cytotoxicity Test

Short term invitro cytotoxicity was performed using Dalton's Lymphoma cells (DLA) and Ehrlich Ascites Carcinoma Cells (ECA). The tumour cell aspirated from the peritoneal cavity of tumor-bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by trypan blue exclusion method. Viable cell suspension (110 cells in 0.1ml) was added to tubes containing various concentrations of the compound and the volume was made up to 1ml using phosphate-buffered saline (PBS). The control tube contained only cell suspension. These assay mixtures were incubated for 3 hours at 37°C. The further cell suspension was mixed with 0.1ml of 1% trypan blue and kept for 23 minutes then loaded on a hemocytometer for cell count. Dead cells take up the blue color of trypan while live cells do not up the dye. The numbers of stained and unstained cells were counted separately.

$$\% \text{ cytotoxicity} = \frac{\text{No. of Dead Cells}}{\text{No. of Live Cells} + \text{No. of Dead Cells}} \times 100$$

RESULTS AND DISCUSSION

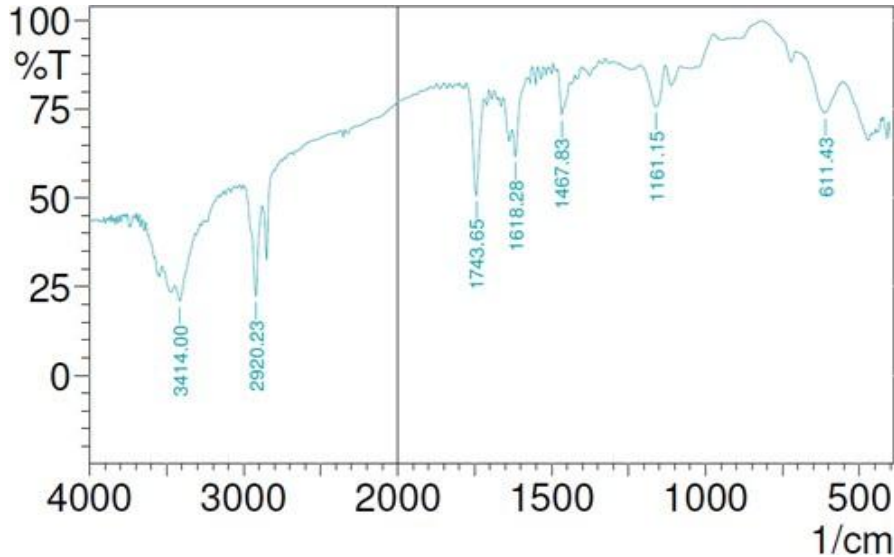
Preliminary Screening of Phytoconstituents of *Biophytumsensitivum Linn*.

The preliminary screening tests of methanol extract showed presence of high concentration Phenols and flavonoids as compared with the petroleum ether. (Table.1). FTIR analysis was used to find out the phytoconstituents type using functional groups. It showed presence of Phenols and Flavonoids. (Fig.2 and Fig.3)

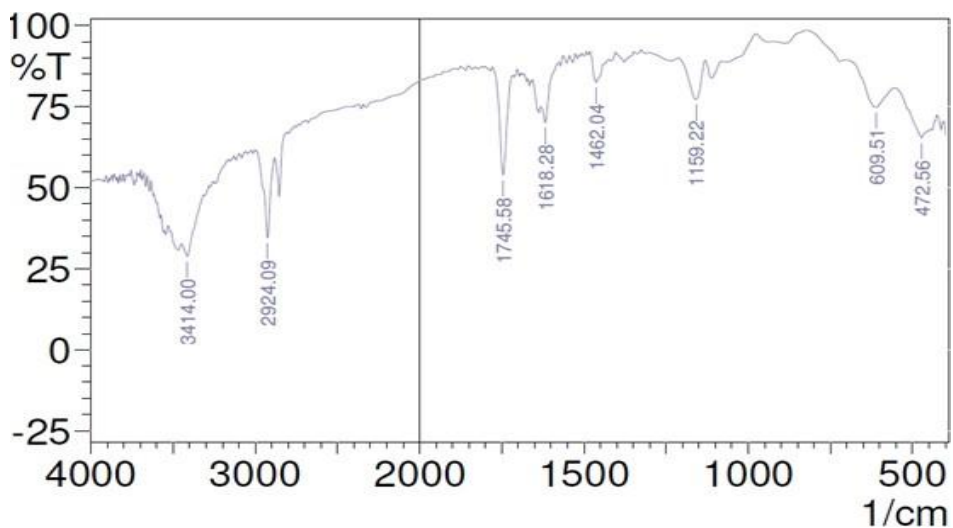
of *Biophytumsensitivum Linn* It showed presence of Phenols and Flavonoids. Alky halides (C O Stretch) peak was observed at 609.51 in methanol extract of *Biophytumsensitivum Linn* (Table.2 and Table.3)

Tab. 2: FTIR Analysis of Petroleum Ether Extract of *Biophytumsensitivum Linn.*

S.No	PEAK	FUNCTIONAL GROUP
1	611.43	Alkyl halides (C Br Stretch), bromide, iodide
2	1161.15	Alkyl halides(C O Stretch), alcohols, ethers, esters, carboxylic acids anhydrides. Amines
3	1467.83	Aromatics(C C Stretch(in-ring))
4	1618.28	Alkenes(C=C Stretch) aldehydes , Saturated aliphatic(C=O Stretch)
5	1743.65	Esters, saturated aliphatic(C=O Stretch) carboxylic acid, carbonyls (general)
6	2920.23	Alkanes (C H Stretch), terminal triple bond, primary, secondary amines.

Fig. 2: FTIR analysis of petroleum ether extract of *Biophytumsensitivum Linn.*Tab. 3: FTIR analysis of methanol extract of *Biophytumsensitivum Linn.*

S.No	PEAK	FUNCTIONAL GROUP
1	472.56	Alkyl halides (C Br Stretch), bromide
2	609.51	Alkyl halides(C O Stretch), alcohols, ethers, esters, carboxylic acids, anhydrides. amines
3	1159.22	Aromatics (C C Stretch (in ring))
4	1462.04	Alkenes(C=C Stretch) aldehyde, Saturated aliphatic(C=O Stretch), Aromatic
5	1618.28	Esters, saturated aliphatic(C=C Stretch) carboxylic acid, carbonyls (general)
6	1745.58	C=O Stretching (ester)
7	2924.09	Alcohols, phenols (O H Stretch, H bonds).
8	3414	N H Stretching

Fig. 3: FTIR analysis of methanol extract of *Biophytumsensitivum Linn.*

Invitro Cytotoxicity Studies**Cytotoxicity Effect in DLA Method**

Serial concentrations (200, 100, 50, 20 and 10 µg/ml) of extracted compound solutions were prepared by using phosphate-buffered saline (PBS). (Table-4) As compared

with petroleum ether extract compound, methanol extract compound showed 100% cell death at the concentration of 200 µg/ml where petroleum ether extract showed 68% cell death in Dalton's Lymphoma Cells.

Tab. 4: Evaluation of invitro anti-cancer activity of *Biophytumsensitivum* Linn. by DLA method.

S. No	Concentration of Extracted Component	% Cell Death	
		Methanol Extracted Component	Petroleum Ether Extracted Component
1	200 µg/ ml	100%	68%
2	100 µg/ ml	71%	52%
3	50 µg/ ml	26%	38%
4	20 µg/ ml	20%	31%
5	10 µg/ ml	12%	23%

Cytotoxicity Effect in ECA method

Serial concentration (200, 100, 50, 20 and 10 µg/ml) of extracted compound solutions were prepared by using phosphate-buffered saline. As compared with petroleum

ether extract compound methanol extract compound showed 100% cell death at the concentration of 200 µl. Where petroleum ether extracted compound showed 72% cell death in Ehrlich Ascites Carcinoma Cells.

Tab. 5: Evaluation of invitro anti-cancer activity of *Biophytumsensitivum* Linn. by ECA.

S.No	Concentration of Extracted Component	% Cell Death	
		Methanol Extracted Component	Petroleum Ether Extracted Component
1	200 µg/ ml	100%	72%
2	100 µg/ ml	76%	58%
3	50 µg/ ml	32%	44%
4	20 µg/ ml	20%	32%
5	10 µg/ ml	10%	21%

CONCLUSION

Nowadays herbal research and ethnopharmacological studies have been carried throughout the world. Traditionally *Biophytumsensitivum* Linn was used widely for various diseases. In the present study, the anti-cancer activity was mainly attributed to the phytoconstituents present in *Biophytumsensitivum* Linn which were confirmed by FTIR stretchings. The study showed a marked proof for non-toxic anti-cancer activity at a dose of 200 µg/ml of methanol extract of *Biophytumsensitivum* Linn using DLA and EAC cell lines. Hence the study was made an attempt to identify a novel plant drug as an alternative to conventional anti-neoplastic drugs. Further specific studies are required to find out the specific individual components.

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