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Research Article

Anti-Tumour Activity of An Ethanolic Extract of the Entire Plant of *Biophytum Sensitivum* from their Pharmacognostical, Pharmacological and Phytochemical Screening a Review

Bal Prijith A.*, Manojkumar P., Logesh K., Mamtha M., Kavi Bharathi R., Kesavan C.

Department of Pharmacognosy, Srinivasan College of Pharmaceutical Sciences

This review investigates the pharmacognostical, phytochemical, and pharmacological profiles of *Biophytum sensitivum* (L.) DC., an ethnomedicinal plant recognized for its diverse therapeutic applications, including potent anti-tumor properties. Phytochemical screening reveals that the whole plant is a rich source of bioactive secondary metabolites such as flavonoids, phenolic compounds, tannins, and saponins, with amentoflavone identified as a major constituent driving its pharmacological efficacy. To assess its pharmacological potential, *in vitro* anti-tumor activity was evaluated using the MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Various solvent extracts (ethanolic, chloroform, petroleum ether, and aqueous) were tested against VERO (normal monkey kidney), HELA (cervical cancer), and HEPG2 (liver cancer) cell lines over 12, 24, and 48-hour intervals. The results demonstrated a dose- and time-dependent anti-proliferative effect, with the ethanolic extract consistently exhibiting the highest cytotoxicity and anticancer activity against both HELA and HEPG2 cell lines at minimal concentrations. Meanwhile, the aqueous extract showed the highest non-toxic concentration in the normal VERO cell line. The findings strongly suggest that *B. sensitivum* contains novel anticancer compounds, positioning it as a highly promising therapeutic candidate for the treatment of liver and cervical cancers.

Keywords: Biophytum sensitivum, Anti-tumor Activity, Cytotoxicity, MTT Assay, HELA Cell Line, Cell Line, Amentoflavone, Phytochemical Screening.

INTRODUCTION

Biophytum sensitivum (L.) DC., is widely recognized for its distinctive touch-sensitive foliage and its broad spectrum of therapeutic applications. *Biophytum* is a genus of about 50 species of annual and perennial herbaceous plants distributed in tropical Asia, Africa, America and Philippines. In India, nine species are found and out of these only three species viz., *Biophytum sensitivum* DC. (Syn *Biophytum petersianum Klotzsch*.), B. *reinwardtii Edgew*. and B. *umbraculum Welw.* are reported to have ethnomedicinal properties. B. *sensitivum* (Family - Oxalidaceae) commonly known as 'Nagbeli' and "Lajjalu", is an annual herb that grows at the foothills of the Himalayas, around the inner Tarai region (east of Koshi river) in Eastern Nepal.

Fig.1

It is a common weed distributed in wet lands (mostly plains) of tropical Africa, Asia and India, and is found normally in the shade of trees and shrubs, in grasslands, at low and medium altitudes. It is commonly known as by various vernacular names such as Lajjalu (Hindi), Sensitive Plant (English), Mukkutti (Malayalam), Alambusha, Jalapushpa, Panktipatra, Pitapushpa (Sanskrit), Nilaccurunki, Tintanali (Tamil), Jhalai (Bengali), Hara Muni Jalapushp (Kannada), Jharera, Lajwanti (Marathi), Attapatti, Chumi, Jala (Telugu) and Alleluya (French). It is easily propagated through seeds. Seeds are propelled away from the plant by built up tension from when they dry and sown in a mixture of moist peat and sand, after sowing it is covered with a transparent cover to increase humidity. It requires bright indirect sunlight to partial shade, medium humidity and 16 °C to 29 °C temperature, moist soil and water-soluble fertilizers during growth season. The ethnomedicinal value of Biophytum sensitivum is profound, with various parts of the plant being used to treat a wide array of ailments, including wounds, inflammation, asthma, diabetes, tumors, and infections. Traditionally, it has been employed as an anti-inflammatory, antioxidant, anti-diabetic, immunomodulatory, and antimicrobial agent. Phytochemical analyses have revealed that Biophytum sensitivum is a rich source of bioactive compounds such as flavonoids, phenolic acids, tannins, terpenoids, saponins, and polysaccharides. Among these, amentoflavone, a biflavonoid compound, has been extensively studied for its potent pharmacological properties. These secondary metabolites are believed to be responsible for the plant's therapeutic potential and are increasingly gaining attention in pharmacognosy and phytopharmacology research.

Components:

The little plant grows up to maximum of 20 cm and possesses unbranched woody erect stem. Leaves: Leaves abruptly pinnate, leaflets opposite, 6 to 12 pairs, and each leaflet is up to 1.5 cm long, the terminal pair is the largest. Flower: The flowers are many and crowded at the apices of the numerous peduncles, normally yellow, white, or orange with red streak in the center of each of the five petals. The sepals are subulate-lanceolate, striate, and about 7 mm long. Fruits: Fruits are ellipsoid capsules which are shorter than the persistent calyx.

Flowers:

Flowers are dimorphic, normally yellow, white or orange with a red / orange streak in the center of each of the 5 petals on long peduncles of various lengths; petals usually twice as long as the sepals, capsules elliptic, shining. The flowers are many, and crowded at the apices of the numerous peduncles. The sepals are subulate-lanceolate, striate, and about 7 millimetres long. Interesting feature of flowers of this plant is heterostyly. Heterostyly in *B. sensitivum* is responsible for 3 flower morphs. The three morphs (tristylous) each have a stable difference in pistil- and stamen length. The fruit is a capsule which is ellipsoid, apiculate, slightly exceeding the sepals. Seeds are ovoid and transversely striate.

Fig.2

Leaves:

Leaves are green in color, peripinnate, 3.7-12.7cm long, crowded into a rosette on the top of the stem; leaflets 6-15 pairs, oblong, very variable in size, 6-12 mm long 5, 13. The remarkable feature of leaflets is their ability to fold together representing an extreme form of "sleep movement" which is exhibited by a lot of members in this family. When applying pressure, tapping or damaging them they fold together in a few seconds. This plant also displays this behavior when the light drops at night. This ability is not restricted to the leaves; the peduncle which carries the flowers has the same ability and also drops at night.

Fig.3

Root:

The plant possesses a slender, fibrous root system that extends superficially into the soil. The roots are typically delicate, light brown, and moderately branched, providing support for the small erect stem. Due to the plant's preference for moist habitats, the roots remain flexible and thin, enabling efficient absorption of water and nutrients.

Fig.4

Stem:

The stem is simple, erect, and unbranched, usually 3–15 cm tall, and cylindrical in structure. It is green to light brown, smooth, and often slightly swollen at the nodes. The leaves are densely crowded into a terminal rosette at the apex of the stem, giving the plant a miniature palm-like appearance.

Fig.5

Seed:

The seeds are small, ovoid to ellipsoidal, and occur inside tiny, dehiscent capsules formed after flowering. Each capsule contains several minute seeds, typically brownish to yellowish in colour. The seeds are lightweight and adapted for short-distance dispersal when the capsule splits open. Upon maturity, the seed coat becomes firm, enabling protection during germination.

Fig.6

Chemical Constituents and Their Activity:

The whole plant contains various chemical constituents like phenolic and polyphenolic compounds, saponin, essential oil, polysaccharides and pectin. The main constituent was found to be amentoflavone. Amentoflavone was quantified by reversed phase high performance liquid chromatography (RPHPLC) in methanolic extract of roots, stems and leaves and the contents were estimated to be 0.26% in roots, 0.33% in stems, and 0.012% in leaves 18. High-performance thin layer chromatographic (HPTLC) method has been developed for estimation of amentoflavone and was

validated for precision (intra- and inter-day), repeatability, and accuracy were 0.52-1.36% 19. Various chemical onstituents of B. sensitivum have been summarized.

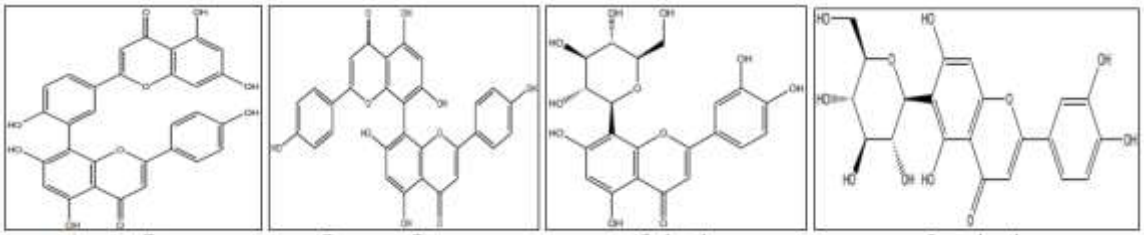
Plant Part:

Aerial parts: Amentoflavone and Cupressoflavone (bioflavone) 20, 21 Polysaccharide, BP100 III, which is composed of galacturonic acid and rhamnose 22, 23 Luteolin-7-methyl ether, isoorientin and 3-methoxyluteolin 7-O-glucoside (Flavonoids) 20 4-caffeoylquinic acid and 5-caffeoylquinic acid 21.

Leaves: Orientin, isoorientin, isovitexin, isoorientin 7-O-glucoside, isoorientin 2-O-rhamnoside 24, 25

Roots: (–)-epicatechin 21.

Whole plant: 1, 4-dimethoxy benzene, 1, 2-dimethoxy benzene, 2-methoxy-4-methyl phenol, (Z)-linalool oxide, (E)-linalool oxide, linalyl acetate, 1-octen-3-ol and isophorone 26.



Medicinal Uses:

Medicinal Use	Chemical Constituents	Site Of Action
Antioxidant	Flavonoids (Amentoflavone, Isoorientin) and Phenolic compounds	Scavenges free radicals, protects against oxidative damage, and increases the activity of antioxidant enzymes.
Anti-inflammatory	Amentoflavone, Procyanidins	Inhibits the production of pro-inflammatory cytokines (like IL-1beta, TNF-alpha) and may downregulate COX-2 expression.
Anticancer / Antitumor	Biflavones (Amentoflavone, Cupressuflavone)	Inhibits tumor growth, induces apoptosis (programmed cell death) in cancer cells, and shows anti-angiogenic effects (inhibiting new blood vessel formation essential for tumor growth).
Antidiabetic	Biflavones, Flavonoids	Helps reduce blood glucose and glycosylated hemoglobin levels, possibly by stimulating insulin synthesis/release.
Immunomodulatory	Extracts and Amentoflavone, Polysaccharides (BP100 III)	Stimulates the immune system, enhances immune cell proliferation, and increases antibody-forming cells.
Radioprotective	Extracts	Protects against damage induced by radiation exposure, potentially by scavenging free radicals and mediating immunomodulation.
Antimicrobial	Extracts (Methanol, Chloroform)	Exhibits antibacterial activity against various pathogens.
Traditional/Other Uses	Overall Phytochemical Profile	Used traditionally for ailments such as wounds and ulcers, asthma, rheumatism/arthritis, insomnia, convulsions, chronic skin diseases, and as an antifertility and antihypertensive agent.

Introduction About the Anti-Tumor Property:

A tumor, also known as a neoplasm, is an abnormal mass of tissue that forms when cells grow and divide more than they should, or do not die when they should. This overgrowth of abnormal cells creates a lump or mass within the body.

Abnormal Growth: The cells multiply in an unregulated, unrestrained, and independent manner, meaning their growth is not controlled by the normal regulatory mechanisms of the body.

Origin: Tumors can form almost anywhere in the body, including organs, tissues, bone, and skin.

Difference from Cysts: A key distinction is that a tumor is a solid mass of tissue, while a cyst is typically a small sac that contains fluid, air, or other non-solid material.

Tumor Classification:

Tumors are primarily classified based on their potential to cause harm, their cellular origin, and their extent of spread.

1. Classification by Behavior (Malignant vs. Benign)

This is the most fundamental way to classify a tumor.

Benign: Non-cancerous. They are localized and do not spread.

* Generally slow-growing.
* Often surrounded by a capsule (well-defined border).
* Do not invade surrounding tissue.
* Rarely life-threatening (unless they press on vital structures).

Malignant: Cancerous. They have the ability to invade tissues and spread.

* Often fast-growing.
* Lack a capsule (irregular, invasive border).
* Invade nearby tissues.
* Can metastasize (spread) to distant parts of the body via the blood or lymphatic system.

Precancerous: Abnormal cells that are not yet cancerous but have the potential to become malignant if left untreated (e.g., dysplasia, carcinoma *in situ*).

* Cells are abnormal and dividing rapidly.

2. Classification by Tissue of Origin (Malignant Tumors):

Malignant tumors (cancers) are broadly grouped according to the type of cell or tissue from which they arise.

- **Carcinoma**- Epithelial cells (cells that cover the body and line organs/glands). Breast, Lung, Colon, Prostate Cancer (most common type of cancer).
- **Sarcoma**- Connective or supportive tissues (bone, cartilage, fat, muscle, blood vessels). Osteosarcoma (bone), Liposarcoma (fat), Leiomyosarcoma (smooth muscle).
- **Leukemia-** Blood-forming tissues (bone marrow, which produces white blood cells). Acute Myeloid Leukemia (AML), Chronic Lymphocytic Leukemia (CLL).
- **Lymphoma-** Cells of the immune system (lymph nodes, spleen, thymus). Hodgkin Lymphoma, Non-Hodgkin Lymphoma.
- **Myeloma**-Plasma cells (a type of white blood cell) in the bone marrow. Multiple Myeloma.
- **Blastoma-** Embryonic or developing cells (most common in children). Retinoblastoma (eye), Medulloblastoma (brain), Nephroblastoma (kidney).

LITERATURE REVIEW

Pharmacognostical Studies:

Shibila T, Johnson M, Revathy I, Narayani M, Utchimahali M. Phytochemical Profile of Biophytum Sensitivum Dc (Oxalidaceae)

Manisha, Kumar Suresh. Pharmacognostic Standardization of Biophytum Sensitivum Dc

Senthamarai R, Vasuki K. Pharmacognostical Studies of Biophytum Sensitivum Dc Stem

Pharmacological Studies:

Sakthivel K. M., Guruvayoorappan C. Biophytum sensitivum: Ancient medicine, modern targets

Abinash C. Bharati, Alakh N. Sahu. Ethnobotany, phytochemistry and pharmacology of Biophytum sensitivum DC

Invitro Studies:

M. P. Shanthi, G. Bupesh, S. Magesh, K. Meenakurmari, K. Saravana N.S. Muthiah. In Vitro Anticancer Activity of Biophytum Sensitivum Whole Plant Extracts Against Cervical and Liver Cancer Cell Lines.

Sirigiri Chandra Kala, Kokkanti Mallikarjuna. In Vitro Analysis of Cytotoxicity and 5-Lipoxygenase Inhibition Activity by Using Callus Extract of Biophytum sensitivum (L) DC.

Source Of the Plant Material:

Synonym- Oxalis sensitiva L, Biophytum cumingii, Biophytum nervifolium, Biophytum poterioides, Toddavaddia sensitive

Biological source- The entire plant of the annual herb *Biophytum sensitivum* (L.) DC.

Family- Oxalidaceae.

Geographical source- India, Africa, South East Asia, China, Myanmar, Thailand, Vietnam, Malaysia, Indonesia, and the Philippines

Habitat- Shady places, wasteland, riverbanks, under damp thickets, moist and open situations, deciduous forests, and open teak forests. It is also found in pasture lands and fallow fields. It is typically found at elevations up to 250 meters, but has been observed on plains up to 1400 meters. It thrives in warm, humid conditions and moist soil, often growing as a common weed in disturbed environments. It prefers filtered or dappled light and does not tolerate direct afternoon sun.

Cultivation- The *Biophytum sensitivum,* or Little Tree Plant, is a striking miniature annual that thrives when its tropical, wet habitat is closely mimicked. Successful cultivation requires bright, indirect light; harsh direct sun will scorch its leaves. The plant is a moisture-lover, so the soil must be kept consistently moist—never soggy, but never allowed to dry out completely. High humidity (70-90% is ideal) is crucial, making it an excellent candidate for a terrarium or growth near a humidifier. It prefers warm temperatures (18°C–26°C / 64°F–80°F) and will drop its delicate, touch-sensitive leaves if stressed by cold or drought. As it is an annual, the easiest way to ensure continuous enjoyment is to collect and sow the seeds it produces after flowering.

Collection- The collection of *Biophytum sensitivum* is primarily focused on seed harvesting for propagation, as the plant is a short-lived annual. While the plant is widely distributed across tropical Asia and Africa, and is classified as Least Concern in many areas, it is extensively used in traditional Ayurvedic and Siddha medicine. Therefore, commercial or large-scale collection, often of the whole plant, should prioritize sustainable harvesting methods to prevent localized depletion, especially since it is also a common weed and is easily grown from seed. For home growers, the collection simply involves gathering the readily available seeds from the bursting capsules of mature plants to ensure a continuous supply for the next growing season.

Pharmacognostical Studies

Macroscopical Studies:

The macroscopical study involves the examination of external morphological features of the leaves of *Biophytum sensitivum*

Type: They are pinnately compound (feather-like).

Colour: Medium to Dark Green

Size: Typically ranges from 3 mm to 15 mm long and 2 mm to 7 mm wide

Shape: Rosette or Clustered

Margin: Entire

Apex: Rounded (or Obtuse)

Surface: Thin and Delicate

Venation: Pinnate (Visible Veins)

Microscopical Studies:

1. Leaf (Leaflet) Microscopy (T.S.)

- **Epidermis:** Upper and lower epidermis with thin cuticle stomata are anisocytic.
- **Mesophyll:** Differentiated into palisade and spongy parenchyma.
- **Pulvinus (leaf base):** Specialized motor cells that enable folding upon touch or light stimulus.
- **Vascular Bundles:** Collateral, surrounded by bundle sheath cells.

2. Root Microscopy (T.S.)

Type: Slender, fibrous root system.

Epidermis: Thin-walled cells with occasional root hairs.

Cortex: Parenchymatous cells with intercellular spaces.

Endodermis: Distinct, with Casparian strips.

Vascular Cylinder: Radial arrangement of xylem and phloem.

3. Stem Microscopy (T.S.)

Epidermis: Single-layered, covered with cuticle; unicellular trichomes present.

Cortex: Parenchymatous cells, some containing calcium oxalate crystals.

Vascular Bundles: Collateral and closed, arranged in a ring.

- **Pith:** Large parenchymatous cells with intercellular spaces.

4. Trichomes

- **Type:** Unicellular, covering epidermis of stem and leaves.
- **Function:** Protective and possibly involved in reducing transpiration.

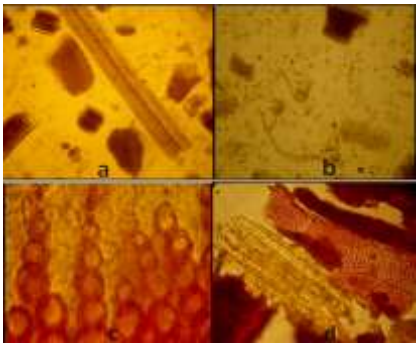


Fig.7 TS of Leaf

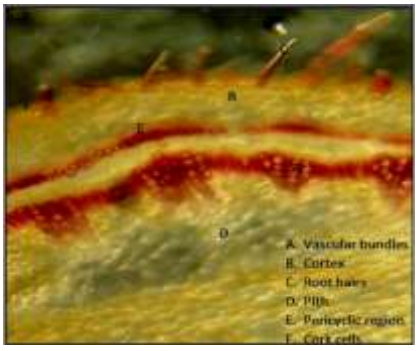


Fig.8 TS of root

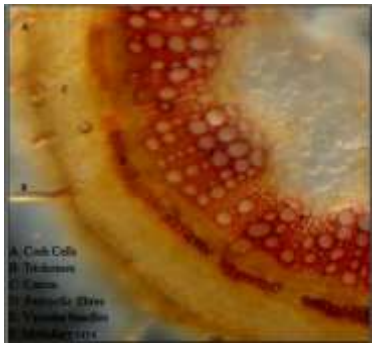


Fig.9 TS of stem

Pharmacological Activity:

B. sensitivum inhibited the growth of solid tumor induced by DLA cells and ascites tumor induced by EAC cells. It also decreased cellular Glutathione (GSH), Serum Gamma Glutamyl Transpeptidase (GGT), and NO levels. This was congruent to the report that the aqueous extract of B. sensitivum leaves showed significant antitumor activity against the transplantable murine tumor.

Chemical Tests:

Test for Flavonoids:

Shinoda Test: Take 2ml plant extract in a test tube. Add a few fragments of Magnesium powder. Add

Conc HCl dropwise. Observe the colour change. The development of an intense Red, Pink, Crimson, is a Positive result, indicating the presence of flavonoids.

Test for Saponins:

Foam Test: Take 2ml of plant extract in a stoppered test tube. Add 5ml of distilled water. Shake the test tube vigorously for 15 seconds. Allow the test tube to stand undisturbed for 15 minutes. The formation of a stable foam layer that persists for more than 15 minutes is a Positive result, indicating the presence of saponins.

Test for Tannins and Phenolic Compounds:

Ferric Chloride Test: Take approximately 1ml of the aqueous or alcoholic plant extract in a test tube. Add 2ml of Ferric Chloride solution (typically a 5% or 1% solution). Shake well and observe the colour change. The appearance of a Blue-black, Greenish-black, or Dark Blue coloration is a Positive result, indicating the presence of tannins and/or other phenolic compounds.

Test for Alkaloids:

Mayer's Test: Take 2ml of plant extract Add a few drops of Mayer's Reagent to the extract. Observe the formation of a precipitate. The formation of Yellowish-White precipitate is a Positive result, indicating the presence of alkaloids.

Test for Carbohydrates / Glycosides:

Molisch's Test: Take 2ml of extract. Add 2 drops of Molisch's Reagent. add about 2ml of Conc H_2SO_4 down the side of the tube, allowing the acid to form a layer beneath the extract. The formation of a Reddish-Violet ring at the junction of the two layers is a Positive result, indicating the presence of carbohydrates and glycosides.

Test for Steroids and Triterpenoids:

Liebermann-Burchard Test: Take 2ml of extract to dryness. Redissolve the residue in a small amount of Chloroform. Add 2ml of Acetic Anhydride to the chloroform solution. Carefully add 1ml of Conc H_2SO_4 side of the test tube to form a lower layer. deep blue-green colour in the upper layer or at the junction is a Positive result, indicating the presence of Steroids. A red, violet colouration is often indicative of Triterpenoids.

Extraction Methods

Authenticated *Biophytum sensitivum* was dried at 45˚C and powdered. Ten grams was stirred overnight in 70% Ethanol (100ml), centrifuged at 10,000 rpm at 4˚C for 10min, and supernatant was collected. Ethanol was removed by evaporation, and yield was 12% (w/w).

Fractionation – The 70% Ethanol Extract Was Suspended in Water Sequentially Partitioned with hexane, dichloromethane, ethyl acetate, butanol, and water. Each Fraction Was Concentrated and Freeze-Dried.

Estimation of Total Phenolic Content:

The TPC is measured spectrophotometrically using the Folin-Ciocalteu (FC) assay.

Principle: The FC reagent (a mixture of phosphotungstic and phosphomolybdic acids) is reduced by phenolic compounds in the presence of an alkaline solution (usually sodium carbonate, Na_2CO_3)

Reaction: This reduction causes the formation of a blue-colored chromophore (phosphotungstate-phosphomolybdenum complex).

Measurement: The intensity of the blue color is directly proportional to the total concentration of phenolic compounds. It is measured using a UV-Visible spectrophotometer at a maximum absorbance wavelength, typically around 760nm.

Standardization: A calibration curve is prepared using known concentrations of a standard phenolic compound, most commonly Gallic Acid. The TPC of the sample is then calculated and expressed as mg of Gallic Acid Equivalents (GAE) per gram of the dry extract. This curve is the crucial reference against which the phenolic content of your *Biophytum sensitivum* extract is quantified, allowing results to be expressed as Gallic Acid Equivalents (GAE).

Gallic Acid Stock Solution Preparation

- **Weighing:** Accurately weigh a precise amount of **Gallic Acid** powder (e.g.,10 mg).

- **Dissolution:** Dissolve the weighed Gallic Acid in a specific volume of the appropriate solvent (usually methanol or distilled water) in a volumetric flask to create a **Gallic Acid Stock Solution** (e.g., 100 mug/mL).

Preparation of Working Standards

- From the stock solution, prepare a series of at least 5 to 7 standard solutions covering a working concentration range that brackets the expected concentration of your sample (e.g., 10, 20, 40, 60, 80, 100 mug/mL).

- **Blank:** Prepare a control tube (the **Blank**) containing only the solvent (methanol) and all the reagents, but **no Gallic Acid**.

Running the Assay on Standards

- Apply the standard Folin-Ciocalteu protocol to all working standard solutions and the blank (as detailed in the previous response).
    - Mix the standard/blank with diluted **Folin-Ciocalteu reagent**.
    - Add **Na2CO3 solution**.
    - Incubate for colour development.

- **Measurement:** Measure the **absorbance** (Y) of each standard solution at 760nm (or 765nm) using the reagent blank to zero the spectrophotometer.

Data Plotting and Regression Analysis

- **Plotting:** Plot the concentration of Gallic Acid (mug/ml) on the **X-axis** against the corresponding absorbance on the **Y-axis**.

- Linear Regression: Perform a linear regression analysis on the data points (Absorbance vs. Concentration) to establish the standard curve equation: $Y = mX + c$
    - **m** is the **slope** (extinction coefficient related to Gallic Acid).
    - **c** is the **Y-intercept**.

- **Validation:** Ensure the line is highly linear, typically with a Coefficient of Determination (R) value of 0.99 or greater.

Extraction yields – the 70% methanol extract yield was 15.93%. fraction yields – hexane (18%), dichloromethane (22%), ethyl acetate (32%) (highest), butanol (15%), and water (13%).

Fraction	Polarity	Typical Yield (% Of Cme)	Key Compounds Concentrated
Hexane	Least Polar	18%	Fatty acids, waxes, non-polar terpenes.
Dichloromethane	Low Polarity	22%	Alkaloids, less polar flavonoids (some amentoflavone), moderately polar compounds.
Ethyl Acetate	Medium Polarity	32%	**Major Biflavonoids (Amentoflavone),** most monomeric flavonoids, and cinnamic acid derivatives (e.g., caffeoylquinic acids).
Butanol	High Polarity	15%	Flavonoid Glycosides (flavonoids attached to sugar molecules), saponin glycosides.
Aqueous Residue	Highest Polarity	13%	Carbohydrates (polysaccharides), amino acids, salts, highly water-soluble polar components.

In Vitro Anti-Tumor Activity

Preparation of whole plant extracts Biophytum sensitivum:

100 Microgram of plant extract was dissolved in the 1 ml DMSO and then 1:3 dilution of test compound was prepared for MTT assay.

Cytotoxicity assay by MTT (3- [4,5-Dimethylthiazol-2-yl] - 2, 5- diphenyltetrazolium Bromide) assay:

The monolayer cells were trypsinized and the cell count was adjusted to 1 lakhs cells/ ml using medium containing 10% newborn calf serum. Pre-incubate cells at a concentration of 1× 106 cells/ml in culture medium for 3 h at 37°C and 6.5% CO2. The cells were seeded at a concentration of 5×104 cells/well in 100 µl culture medium and incubated at 37°C in 5% CO2 incubator for 24 hrs. After 24 hours, when the monolayer formed, the supernatant was flicked off and added previously diluted with media of 100µl of different concentrations of Biophytum sensitivum extracts in microtitre plates and kept for incubation at 37°C in 5% CO2 incubator for 72 hour and cells were periodically checked for granularity, shrinkage, swelling. After 72 hours, the sample solution in wells was flicked off and 10µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37°C in 5% CO2 incubator. The supernatant was removed and 100 µl of Dimethyl Sulfoxide (DMSO) was added and the plates were gently shaken to solubilise the formed formazan. The absorbance was measured using a microplate reader at 540 nm with a reference filter of 620 nm 14-17. The percentage of cell growth inhibition or percentage cytotoxicity was calculated by following formula

$$\%\ \ of\ Growth\ \ Inhibition\ \ = \left[\frac{100 - \text{Mean OD of Test Group}}{\text{Mean group of Control Group}}\right] \times 100$$

MTT assay performed for antiproliferative activity VERO, HELA and HEPG2 cell lines. The VERO cell line is initiated from the kidney of a normal adult African green monkey. This cell line was screened to evaluate the cytotoxicity effect in normal cell line. The Liver cell line HEPG2 is the perpetual cell line which was derived from the liver tissue of a 15-year-old Caucasian American male with a welldifferentiated hepatocellular carcinoma. This cell line was chosen to evaluate the anticancer activity of Biophytum sensitivum in Liver cancer

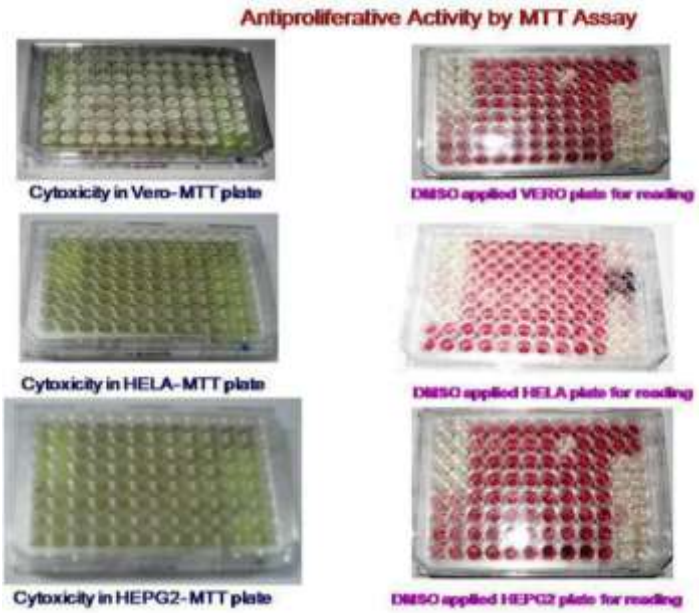


Fig.10

The antiproliferative activity of different extracts of Biophytum sensitivum in HELA cells. The ethanolic extract showed highest anticancer activity than Chloroform, Petroleum ether and aqueous extracts. The ethanolic extract possess anticancer activity at minimum concentration at 40µg, Chloroform extract at 60µg, Petroleum ether at 75µg, and Aqueous extract at 100µg. The cytotoxicity of Biophytum sensitivum extracts were screened at different time intervals viz, 12, 24 and 48 hrs. The ethanolic extract showed higher cytotoxicity in 12 hrs. interval than all the extracts. The cytotoxicity of ethanolic extract attains notable activity at dose dependent manner. The

Chloroform extracts exhibited significant effect of anticancer activity than Petroleum ether and aqueous extract. The anticancer activity of different extracts of Biophytum sensitivum attains in the order of Ethanol< Chloroform < Petroleum ether < aqueous.

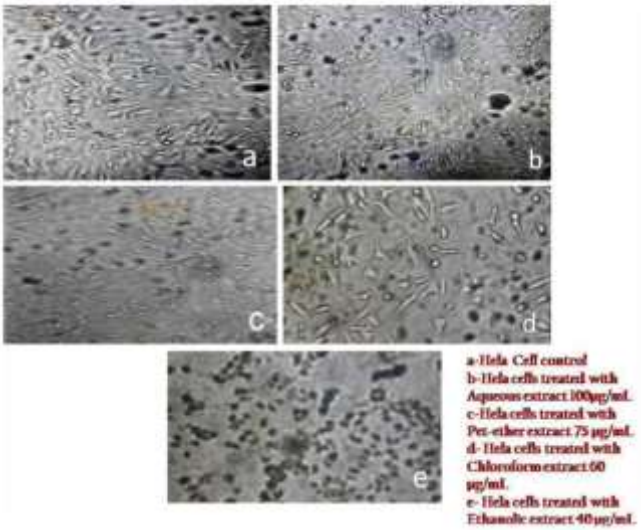


Fig.11

The anticancer activity of different extracts of Biophytum sensitivum in HEPG2 Cells. The ethanolic extracts exhibits HEPG2 anticancer activity at minimum concentration of 30µg, chloroform extract (40µg), petroleum ether extract at (65µg) and aqueous extract at (80µg). The ethanolic extract illustrates higher cytotoxicity in 12 hrs. interval and gradually increases up to 48 hrs. interval in all the extract. The cytotoxicity of ethanolic extract attains higher activity than all the extracts. All the extracts exhibit gradual increase in antiproliferative activity with respect to dose dependent manner. The Chloroform extracts possess significant effect of anticancer activity than Petroleum ether and aqueous extract. The anticancer activity of different extracts of Biophytum sensitivum in HEPG2 attains in the order of Ethanol< Chloroform < Petroleum ether < aqueous.

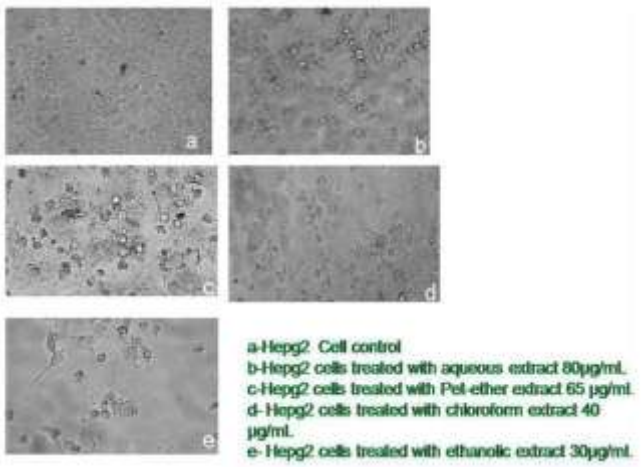


Fig.12

The minimum concentrations of anticancer activity in Biophytum sensitivum various extract were screened. The ethanolic extract possess anticancer activity at 65 µg as minimum concentration, chloroform extract attains 85 µg, Petroleum ether extract attains 100 µg. The normal cell line Vero showed maximum nontoxic concentration upto 150 µg/mL in aqueous extract than other extracts. The ethanolic extract illustrates higher cytotoxicity in 12 hrs. interval and gradually increased at 48 hrs. interval in all the extract. But the

cell viability was pronouncedly increased in the aqueous and petroleum ether extract for all the cell line at lower concentrations.

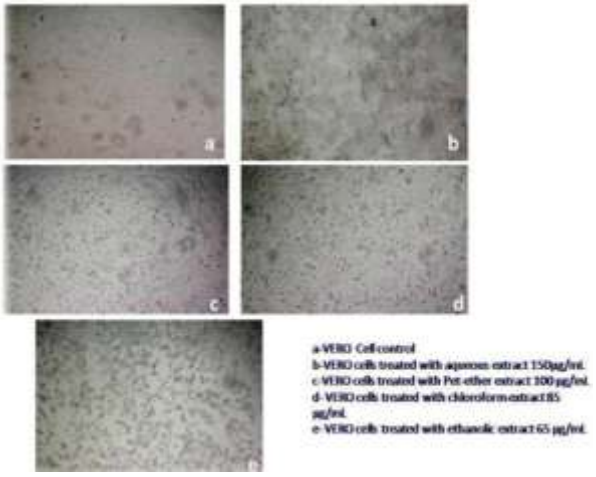


Fig.13

RESULTS:

Chemical Test:

Chemical tests were identifying the phytochemicals as described Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Name of test	Test applied / Reagent used	Ethanol	Water	Chloroform	Hexane	Acetone	Ethyl acetate
Alkaloids	A] Mayer's	+++	++	++	++	+++	++
	B] Wagner's	+++	++	++	++	+++	++
	C] Hagner's	+++	++	++	+++	+++	++
	D] Dragndorff's test	++	++	++	++	++	+
Flavonoids	HCl and magnesium turnings	+++	++	+	++	+	++
Carbohydrate	Molisch's test	+	+	+	+	+	+
Tannins & phenols	A] 10% Lead acetate	+++	+	++	++	++	++
	B] $Fecl_3$	+++	+	++	++	++	++
Test for steroids	A] Salkowski's test	++	++	++	++	++	++
	B] Libermann-Burchard's test	++	++	++	++	++	++
Gums & mucilages	Alcoholic precipitation	-	-	-	-	-	-
Fixed oil & fats	Spot test	+	-	+	+	-	-
Saponins	Foam test	+	+	+	+	+	+
Phytosterols	LB test	+	+	+	+	+	+
Volatile oils	Hydro distillation method	+	+	+	+	+	+
Protein & free amino acids	A] Biuret test	++	++	++	++	++	++
	B] Ninhydrin test	+++	++	++	++	++	++
	C] Xanthoprotein test	+++	++	++	++	++	++

Extraction Yields:

70% methanol extract yield was 15.93%. fraction yields – hexane (18%), dichloromethane (22%), ethyl acetate (32%) (highest), butanol (15%), and water (13%)**.**

Fraction	Polarity	Typical Yield (% Of Cme)
Hexane	Least Polar	18%
Dichloromethane	Low Polarity	22%
Ethyl Acetate	Medium Polarity	32%
Butanol	High Polarity	15%
Aqueous Residue	Highest Polarity	13%

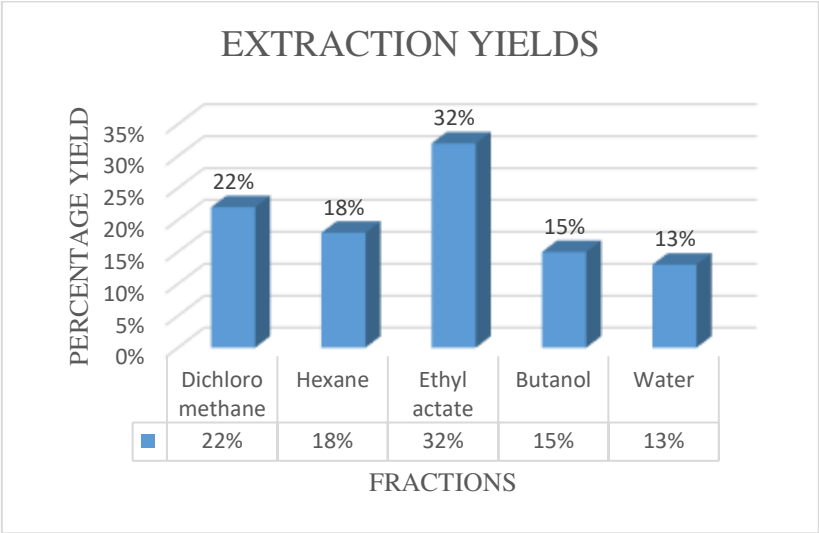


Fig.14

Estimation Of Total Phenolic Content:

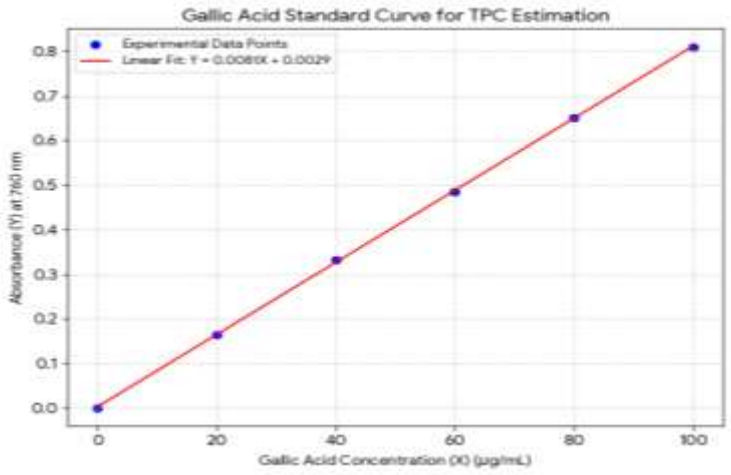


Fig.15

Invitro Studies

Cytotoxicity assay by MTT (3- [4,5-Dimethylthiazol-2-yl] - 2, 5- diphenyltetrazolium Bromide) assay:

The Biophytum sensitivum (B.S) plant extracts were evaluated for the anticancer activity. The extract was screened against the cell line such as HELA, VERO & HEPG2. Finally, the present study suggests that the methanolic extract of B.s plant comprised novel anticancer compounds which will be potent therapeutic candidate for liver and cervical cancers.

Table 1: Cytotoxicity (Cell Viability %) of *Biophytum sensitivum* Extracts in VERO Cells

Time	Extract	20 µg	40 µg	60 µg	80 µg	100 µg
12 h	Aqueous	90	85	80	60	50
12 h	Petroleum ether	80	70	60	50	45
12 h	Chloroform	50	40	30	40	35
12 h	Ethanolic	45	35	25	30	28

24 h	Aqueous	85	60	45	35	30
24 h	Petroleum ether	72	52	42	30	28
24 h	Chloroform	45	38	35	25	23
24 h	Ethanolic	38	30	25	15	13
48 h	Aqueous	75	55	42	35	30
48 h	Petroleum ether	65	45	35	25	20
48 h	Chloroform	40	30	25	20	15
48 h	Ethanolic	32	25	20	15	10

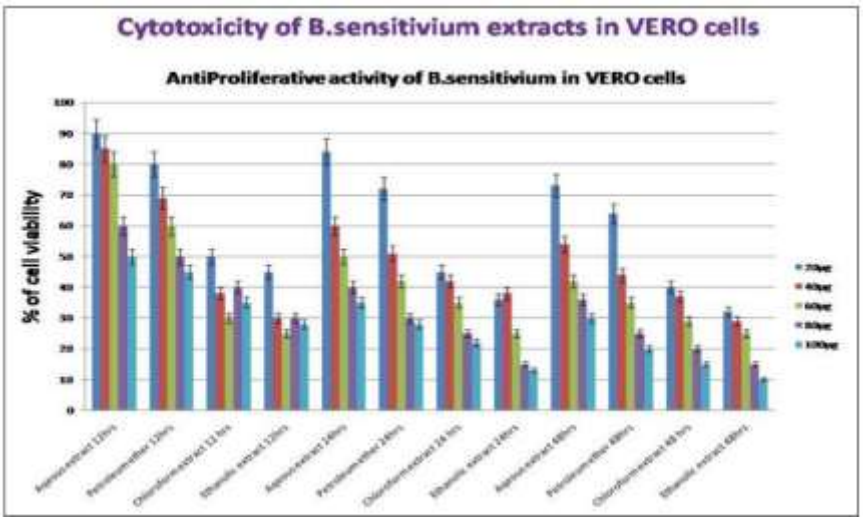


Fig.16

Cytotoxicity of biophytum sensitivum extracts on vero cell line at different intervals

Table 2: Cytotoxicity of *B. sensitivum* Extracts in HeLa Cells (% Cell Viability)

Time	Extract	20 µg	40 µg	60 µg	80 µg	100 µg
12 h	Aqueous	95	90	75	70	60
12 h	Petroleum ether	90	85	78	60	55
12 h	Chloroform	75	70	60	50	40
12 h	Ethanolic	65	55	45	38	35
24 h	Aqueous	90	85	70	60	50
24 h	Petroleum ether	85	78	65	55	45
24 h	Chloroform	55	45	35	25	22
24 h	Ethanolic	45	38	28	18	15
48 h	Aqueous	62	55	40	35	28
48 h	Petroleum ether	55	45	35	25	22
48 h	Chloroform	48	38	28	20	15
48 h	Ethanolic	38	30	22	15	10

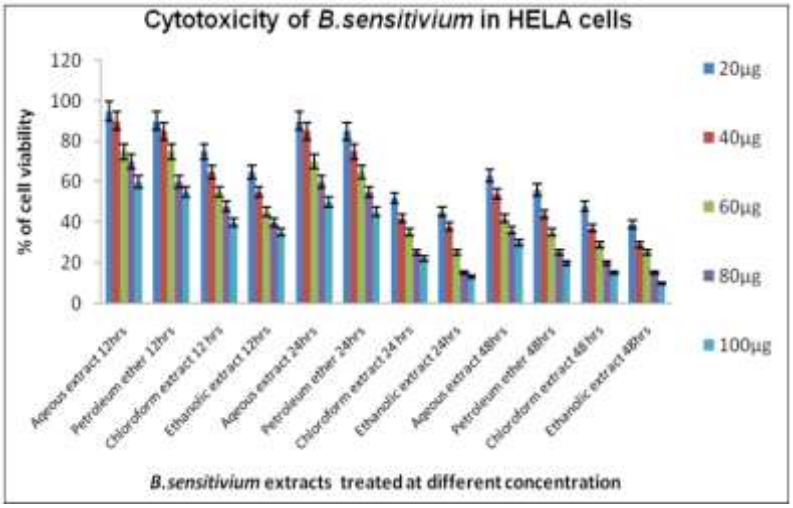


Fig.17

Cytotoxicity of biophytum sensitivum extracts on hela cells at different intervals

Table 3: Cytotoxicity of *B. sensitivum* Extracts in HepG2 Cells (% Cell Viability)

Time	Extract	20 µg	40 µg	60 µg	80 µg	100 µg
12 h	Aqueous	65	60	55	45	35
12 h	Petroleum ether	55	50	45	40	35
12 h	Chloroform	50	45	40	35	30
12 h	Ethanolic	45	30	25	20	18
24 h	Aqueous	60	50	40	35	28
24 h	Petroleum ether	48	42	38	28	25
24 h	Chloroform	35	32	28	15	10
24 h	Ethanolic	30	26	18	10	8
48 h	Aqueous	50	45	38	30	25
48 h	Petroleum ether	45	38	30	22	20
48 h	Chloroform	25	22	18	15	12
48 h	Ethanolic	20	18	15	10	5

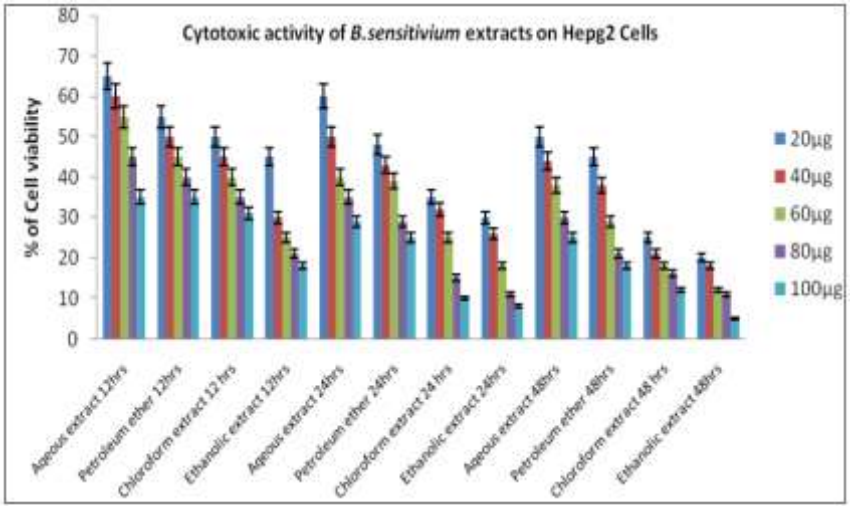


Fig.18

Cytotoxicity of biophytum sensitivum extracts on hepg2 cells at different intervals

DISCUSSION:

The present study focused on evaluating the antitumor potential of various extracts of *Biophytum sensitivum* against different cell lines. Phytochemical screening of the plant revealed a rich profile of bioactive secondary metabolites, including flavonoids (specifically amentoflavone), phenolic compounds, saponins, tannins, and steroids. These compounds, particularly amentoflavone and other biflavonoids, are known for their ability to inhibit tumor growth and induce apoptosis. The extraction process showed that ethyl acetate yielded the highest percentage (32%) among the fractions, indicating that many of the plant's constituents have medium polarity. The in vitro cytotoxicity was evaluated using the MTT assay across three cell lines: HELA (Cervical Cancer), HepG2 (Liver Cancer), and VERO (Normal African Green Monkey Kidney cells). The findings indicate:

- Dose and Time Dependency: The antiproliferative activity for all extracts increased in a dose-dependent (20 µg to 100 µg) and time-dependent (12h to 48h) manner.

- Extract Potency: The ethanolic extract consistently demonstrated the highest anticancer activity compared to chloroform, petroleum ether, and aqueous extracts. For instance, in HepG2 cells at 48 hours, the 100-µg dose of ethanolic extract reduced cell viability to just 5%, compared to 25% for the aqueous extract.

- Cell Line Sensitivity: HepG2 cells appeared more sensitive to the extracts than HeLa cells. The ethanolic extract achieved significant cytotoxicity in HepG2 at a minimum concentration of 30 µg.

- Selectivity and Safety: The VERO normal cell line showed higher tolerance, with maximum non-toxic concentrations reaching up to 150 µg/mL for the aqueous extract. This suggests a level of selectivity where the extracts are more toxic to cancerous cells than to normal cells at similar concentrations.

The order of anticancer potency observed was Ethanol > Chloroform > Petroleum ether > Aqueous. The high activity of the ethanolic extract is likely due to the efficient extraction of potent biflavonoids like amentoflavone, which has been previously quantified in higher amounts in the stem and roots.

CONCLUSION:

The study successfully characterized the pharmacognostical and pharmacological properties of *Biophytum sensitivum*. The results provide strong evidence that the plant, particularly its ethanolic extract, possesses significant antitumor and antiproliferative activity against liver (HepG2) and cervical (HeLa) cancer cell lines. The presence of amentoflavone and other phenolic compounds likely mediates these effects by inducing cytotoxicity in a dose-responsive manner while maintaining a relatively safer profile for normal cells. These findings support the traditional ethnomedicinal use of *Biophytum sensitivum* in treating tumors and suggest that the methanolic and ethanolic extracts contain novel bioactive compounds. Future research should focus on isolating these specific bioactive molecules and conducting in vivo studies to further validate their efficacy and safety as potential therapeutic candidates for cancer treatment.

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