Biological Forum – An International Journal **15(6): 1020-1029(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Evaluation of *in vitro* Anti-cancer Potential of *Macrosolen parasiticus* Stem

Rekha Kanwar*
Mahesh Nagar, Kartarpura Phatak, Jaipur (Rajasthan), India.

(Corresponding author: Rekha Kanwar)*
(Received: 11 April 2023; Revised: 15 May 2023; Accepted: 23 May 2023; Published: 15 June 2023)
(Published by Research Trend)

ABSTRACT: Cancer remains a significant global health challenge, necessitating the search for new and effective therapeutic agents. This study evaluates the in vitro anticancer potential of *Macrosolen parasiticus* stem extracts by assessing its phytochemical profile, cytotoxic effects. Phytochemical screening of different extracts revealed the presence of alkaloids, carbohydrates, phytosterols, fixed oils and fats, phenolic compounds, tannins, proteins, and flavonoids in varying proportions. The cytotoxicity of the extracts was evaluated using brine shrimp lethality bioassay and in vitro assays on Ehrlich Ascites Carcinoma (EAC) cells, MCF-7 (human breast adenocarcinoma), U343 (human neuronal glioblastoma), and A549 (human lung adenocarcinoma) cells through MTT and SRB assays. The aqueous extract exhibited a lower LC50 value (90 µg/ml) compared to the ethanolic extract (120 µg/ml) in the brine shrimp lethality bioassay, indicating a higher cytotoxic potential. In vitro assays demonstrated that both ethanolic and aqueous extracts exhibited significant cytotoxic effects against cancer cells, with dose-dependent cytotoxicity observed across all cell lines. The aqueous extract displayed higher cytotoxicity in comparison to the ethanolic extract, with notable effects against MCF-7, U343, and A549 cells.

Keywords: anti-cancer, *Macrosolen parasiticus,* MTT assay, SRB assay.

INTRODUCTION

Cancer remains one of the most devastating diseases worldwide, characterized by uncontrolled cell proliferation, invasion, and metastasis (Mareel and Leroy 2003). Despite significant advancements in oncology, the disease continues to pose a global health challenge, necessitating the exploration of novel therapeutic agents. Conventional cancer treatments, including chemotherapy, radiation therapy, and targeted drug therapies, often have limitations such as toxicity, resistance, and adverse side effects. As a result, there has been a growing interest in identifying new anticancer compounds, particularly from natural sources, due to their bioavailability, minimal toxicity, and potential efficacy (Kumar *et al.*, 2023; Pooja *et al.*, 2023).

Natural products derived from plants have historically been an important source of bioactive compounds for cancer therapy (Patil *et al.*, 2009). Many well-established anticancer drugs, such as paclitaxel, vinblastine, and camptothecin, are derived from plant sources. The exploration of medicinal plants for anticancer properties is a promising approach, as they contain diverse phytochemicals with potential therapeutic benefits (Shukla & Mehta 2015). One such plant of interest is *Macrosolen parasiticus*, a hemiparasitic plant with traditional medicinal value.

Macrosolen parasiticus (L.) Danser, belonging to the Loranthaceae family, is a medicinal plant commonly found in tropical and subtropical regions. This plant is known for its hemiparasitic nature, deriving nutrients from its host plants while also performing photosynthesis (Těšitel *et al.*, 2010). It has been widely used in traditional medicine, particularly in Asian countries, for treating various ailments, including inflammation, fever, and infectious diseases.

Studies have suggested that *Macrosolen parasiticus* possesses bioactive compounds with potential pharmacological effects, including antioxidant, antimicrobial, and anti-inflammatory activities. However, its anticancer potential remains largely unexplored. Given the increasing focus on natural anticancer agents, evaluating the in vitro anticancer potential of *Macrosolen parasiticus* stem extracts is essential for understanding its role in cancer treatment.

The therapeutic effects of *Macrosolen parasiticus* are attributed to its rich phytochemical composition (Sharath & Naika 2022). Preliminary phytochemical studies have identified the presence of flavonoids, alkaloids, tannins, phenolics, and terpenoids, which are known for their bioactivity. Among these, flavonoids and phenolic compounds have been extensively studied for their antioxidant and anticancer properties (Galati & O'brien 2004). These bioactive molecules have been reported to induce apoptosis, inhibit cancer cell proliferation, and modulate various signaling pathways involved in tumor growth and metastasis.

Despite its traditional medicinal uses, the anticancer potential of *Macrosolen parasiticus* has not been extensively studied. The few existing reports focus on its antimicrobial and antioxidant activities, leaving a significant gap in understanding its role in cancer

therapy. This study aims to bridge this gap by evaluating the in vitro anticancer activity of *Macrosolen parasiticus* stem extracts against human cancer cell lines.

The key objectives of this study include:

- **Phytochemical Screening:** Identifying and quantifying the bioactive compounds present in *Macrosolen parasiticus* stem extracts.
- **Cytotoxicity Assessment:** Evaluating the cytotoxic effects of the extracts on various human cancer cell lines using MTT and other viability assays.

The findings from this study could have important implications for cancer research and drug discovery (Houck & Kavlock 2008). If *Macrosolen parasiticus* demonstrates significant anticancer activity, it could serve as a potential candidate for further investigation in preclinical and clinical trials.

PHARMACOGNOSTICAL STUDY

Materials. Leica DMLS microscope attached with Leitz MPS 32 camera, glass slide, digital electronic balance (Dhaus Corp), cover slip, grinding mixer, stage micrometer, hot air oven (Osworld), watch glass, sonicator (Lequitron), compound microscope, silica crucible, UV apparatus, ashless filter paper (Whatman no.44), magnetic stirrer, petridish, alcohol (95%), stoppered conical flask, chloroform water, sodium hydroxide, chloral hydrate solution, petroleum ether, phloroglucinol, acetone, hydrochloric acid, benzene, sudan red-III, chloroform, ruthenium red, and water.

Source of plant. The plant material was collected from around Maharashtra, India during the month of September. The plant was authenticated and a voucher specimen has been deposited.

Anatomical study. To get rid of the coloring material, free-hand portions of the stem were extracted and heated with chloral hydrate (Lux *et al.,* 2005). After that, clear and homogeneous sections were chosen, stained with HCl and phloroglucinol, placed on a glycerin-sanitized glass slide, and covered with a cover slip. After that, the sections were seen at low power (10 X) and high power (40 X).

Photomicrography. The microphotographs were taken using Olympus BX 41 microscope, attached with Olympus DP20 camera (Ng *et al.,* 2011).

Powder analysis. The microscopic characteristics of the M. prasiticus stem powder were investigated. To remove coloring material, the powder was cooked with chloral hydrate after passing through sieve number 60 (Bunn *et al.,* 1935). It was then examined under a microscope to look for calcium oxalate crystals and other characteristics. For the lignified structures, such as stone cells, the cleared powder was subsequently stained with phloroglucinol in the presence of hydrochloric acid and examined under a microscope as previously mentioned (Whitehill *et al.,* 2016).

Physicochemical Constants

A. Ash values. The residue left over after burning is typically regarded as the crude drug's ash content (Ahmed & Hasan 2015). In addition to inorganic materials added for the aim of adulteration, it represents the inorganic salts that are naturally present in the medicine and adhere to it.

The residue left over after burning is known as total ash. The portion of total ash that is insoluble in diluted hydrochloric acid is known as acid insoluble ash (Himesh *et al.,* 2011). The portion of total ash that dissolves in hot water is known as water-soluble ash.

1. Total ash. In a tared silica crucible, around 2 g of the powdered medication was precisely weighed. A thin layer of the drug powder was applied to the crucible's bottom. Until the crucible was carbon-free, it was burned at a temperature of no more than 450°C. After cooling, the crucible was weighed. Until a consistent weight was noted, the process was repeated. With reference to the air-dried medication, the percentage of the total ash was computed three times.

2. Acid insoluble ash. For five minutes, the ash that was collected according to the procedure for calculating total ash was heated with 25 milliliters of diluted hydrochloric acid (Gul & Safdar 2009). Filtration was used to gather the insoluble ash on ashless filter paper, which was then cleaned with hot water. After being moved into a tared silica crucible, the insoluble ash was burnt, allowed to cool, and then weighed (Neat & Berk 954). Until a consistent weight was noted, the process was repeated. The air-dried medication was used to calculate the proportion of acid-insoluble ash.

3. Water soluble ash. 25 milliliters of water were used to boil the ash that was produced as specified in the total ash determination for five minutes (Etiegni & Campbell 1991). After being collected on ashless filter paper, the insoluble material was cleaned with hot water. After being moved into a tared silica crucible, the insoluble ash was burned at a temperature of no more than 450°C. Until a consistent weight was noted, the process was repeated (Michalek & Papalambros 2005). The weight of the whole amount of ash was deducted from the weight of the insoluble substance. Water-soluble ash was defined as the weight difference. Using the air-dried medication as a reference, the proportion of water-soluble ash was determined.

B. Extractive values. The drug's nature and content are gauged by its extractive value. Different kinds of extractive values exist (Camp *et al.,* 2012).

1. Maceration

(a) Ethanol soluble extractive. A stoppered flask containing five grams of previously weighed air-dried medication was filled with 100 milliliters of 95% ethanol. For seven days, it was constantly shaken on a magnetic stirrer (Muller *et al*., 2005). After that, it was quickly filtered while being careful not to lose the solvent. In a tared, flat-bottomed petri dish, 25 milliliters of filtrate were evaporated till dry, dried at 105°C, and weighed. Using the air-dried medication as a reference, the percentage of ethanol soluble extractive was determined.

(b) Water soluble extractive. 100 ml of chloroform: water (9:1) was added to five grams of previously weighed, air-dried medication in a stoppered flask. For seven days, it was constantly shaken on a magnetic stirrer. After that, it was quickly filtered while being careful not to lose the solvent. In a tared, flat-bottomed

petri dish, 25 milliliters of filtrate were evaporated till dry, dried at 105°C, and weighed. Using the air-dried medication as a reference, the percentage of water-soluble extractive was determined.

C. Determination of moisture content. A surplus of water in medicinal plant materials will promote the growth of microorganisms, the presence of insects or fungi, and degradation after hydrolysis. As a result, water content limits ought to be established for each type of plant. This is particularly crucial for materials that readily absorb moisture or degrade when exposed to water.

D. Loss on drying (LOD). A flat weighing vial that had been previously dried and tared was used to precisely weigh 2–5 g of the prepared air-dried material. After being equally spread, the sample was put in the oven to dry. The bottle was taken out of the oven, quickly closed, and allowed to cool to room temperature before being weighed. The drying process involved heating the bottle to between 100 and 105°C. Unless the test technique specified otherwise, the experiment was repeated until two consecutive weigh-ins did not deviate by more than 5 mg. After drying, the weight reduction was computed.

E. Foaming index. When an aqueous decoction is agitated, saponins included in many therapeutic plant materials can produce persistent froth. A foaming index was created to gauge how well plant materials and their extracts foamed when aqueously decocted.

Procedure. After precisely weighing one gram of the coarse powder (sieve size 1250), it was put into a 500 ml conical flask with 100 ml of boiling water and kept there for 30 minutes. After cooling and filtering the mixture, enough water was added to a 100 ml volumetric flask to reach the desired volume. Ten stoppered test tubes were filled with the aforementioned decoction in sequential quantities of 1, 2, 3, and up to 10 ml. The volume of the liquid was then adjusted in each tube with water to reach 10 ml. After stopping the tubes and shaking them lengthwise for 15 seconds at two frequencies per second, they were left to stand for 15 minutes, and the height of the foam was measured. The following is how the results were evaluated:

- The foaming index was less than 100 if the foam height in each tube was less than 1 cm.
- The volume of the plant material decoction in tube (a) was utilized to calculate the index if the height of foam in any tube was measured to be 1 cm. For a more accurate result, an intermediate dilution was made if this tube was the first or second in a series.
- The foaming index exceeded 1000 when the foam height in each tube exceeded 1 cm. In this instance, a fresh sequence of dilutions of the decoction must be determined in order to get a result.

Foaming Index = 1000/a

Where a, is the volume in ml of the decoction used for preparing the dilution in the tube where foaming was observed.

F. Tannin Content. By interacting with proteins to generate water-soluble molecules that are resistant to proteolytic enzymes, tannins are chemicals that can transform animal hide into leather. This technique is employed as a medicinal agent because it is known to be astringent when administered to living tissue.

Procedure. A conical flask was filled with roughly 2 g of the drug powder after it had been weighed. After adding 150 ml of water, it boiled for 30 minutes. After cooling, it was moved to a 250 ml volumetric flask and filled with water to reach the desired volume. After filtering the solution and drying 50 ml of the extract to dryness, the total amount of material extractable into water was calculated. The drying process was then repeated at 105º C until a consistent weight was achieved, as shown in T1. It was established how much plant material was extractable into water and how much remained unbound to the hide powder after its inclusion. About 80 milliliters of the aforementioned extract were mixed with 6.0 grams of hide powder. After 60 minutes of shaking, the mixture was filtered. A consistent weight (T2) was achieved by drying 50 milliliters of the filtrate at 105 degrees Celsius. In order to ascertain the solubility of hide powder, 6.0 g of hide powder was mixed with 80 ml of water, agitated for 60 minutes, and then filtered. As previously said, 50 milliliters of clear filtrate were evaporated until they were dry, and this was recorded (T0). Thus, the following formula was used to determine the amount of total tannins.

Quantity of tannins (%) = $[T_1 – (T_2 + T_0)] \times 500 / w$

Where w, is the weight of stem powder in grams

G. Determination of swelling index. A 25 ml glass stoppered measuring cylinder was filled with 1 g of the powdered material. 25 ml of water was then added, and the mixture was shaken well every 10 minutes for one hour. It was shaken and then left to stand at room temperature for three hours. It was measured how many milliliters (ml) the plant material, including sticky stuff, occupied.

Phytochemical Study

Materials, instruments and chemicals

Chromatographic paper, TLC plates, china dishes, beakers, conical flasks, TLC chambers, sprayers, digital electronic weighing balances, desiccators, distillation apparatus, test tubes, and soxhlet apparatus were all utilized. All of the chemicals and reagents utilized came from Central Drug House Pvt. Ltd. in New Delhi, Fischer Inorganics & Aromatics Ltd. in Madras, NICE Chemicals Ltd. in Cochin, and Ranbaxy Fine Chemicals Ltd. in Punjab.

Preliminary phytochemical screening

Preparation of extracts

A. Petroleum ether extract. 50 g of M. prasiticus's coarsely dried stem powder was extracted using a hot extraction method (soxhlet) and petroleum ether at 60–80°C. Following the extraction process, the solvent was extracted using distillation and then concentrated in a vacuum.

B. Benzene extract. Following the drying of the marc left over from the extraction of petroleum ether, benzene was extracted using a hot extraction method (soxhlet). Following the extraction process, the solvent was extracted by distillation and then concentrated in a vacuum.

C. Chloroform extract. Following benzene extraction, the remaining marc was dried and extracted using chloroform using a hot extraction method (soxhlet). Following the extraction process, the solvent was extracted using distillation and then concentrated in a vacuum.

D. Acetone extract. Following the chloroform extraction, the remaining marc was dried and removed using acetone using a hot extraction method (soxhlet). Following the extraction process, the solvent was extracted using distillation and then concentrated in a vacuum.

E. Ethanol extract. Following acetone extraction, the remaining marc was dried and extracted using a hot extraction method (soxhlet) with 95% ethanol. Following the extraction process, the solvent was extracted using distillation and then concentrated in a vacuum.

F. Aqueous extract (Chloroform: water – 1:99). Following drying and macerating the ethanolic extraction with chloroform water, the marc remained. Following the extraction process, the solvent was extracted using distillation and concentrated in a vacuum.

Studies on phytochemistry were conducted using the aforementioned extracts. Each extract's extractive values were determined and noted.

Phytochemical analysis of extract of stem of *M. parasiticus*

The following chemical analyses were performed to determine the presence of distinct phytochemical elements in various extracts of M. parasiticus stem.

— Test for saponins. Heamolytic test, Foam test.

— Test for steroids. Salkowaski test, Libermann test, Libermann-Burchard test.

— Test for alkaloids. Hager's test, Wagner's test, Dragendroff's test, Mayer's test.

— Test for glycosides. Liebermann's test, Legal's test, Baljet's test, Modified Borntrager's test, Borntrager's test.

— Test for carbohydrates. Benedict's test, Fehling's test, Molisch's test

— Test for tannins and phenolic compounds. Lead acetate, Dilute ferric chloride, Aqueous bromine solution, Gelatin.

— Test for fixed oils and fats. Saponification test, Spot test

— Test for gums and mucilage. Molisch's test, Precipitation with absolute alcohol.

— Test for proteins and amino acids. Ninhydrin test, Biuret test

— Test for flavonoids. Shnoda's test

ESTIMATION OF TOTAL PHENOLIC CONTENT

Reagents. Folin Ciocalteu Reagent and Sodium Carbonate (20 % w/v)

Procedure. A 25 ml volumetric flask was filled with a stock solution containing 1 mg/ml of aqueous and methanolic extract, together with 1.5 ml of Folin Ciocalteu's reagent and 10 ml of water. Five minutes later, four milliliters of a 20% w/v sodium carbonate solution were added, and the volume was adjusted to twenty-five milliliters using double-distilled water. After 30 minutes, the absorbance was measured at 765 nm. Using the same method, the calibration curve of gallic acid (50-250 µg) was plotted to determine the percentage of total phenolics. Total phenolics were then expressed as milligrams of gallic acid per dry weight of extract.

Total Flavonoid Content

Reagents. Potassium acetate (1 M), Aluminium chloride (10%)

Procedure. Flavonoids were determined using the colorimetric technique using aluminum chloride. Methanol was used to dissolve 10 mg of quercetin, which was further diluted to 25, 50, and 100 µg/ml. Separately, 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water were combined with the diluted standard solution (0.5 ml). A Shimadzu UV-1650PC UV-Visibal spectrophotometer was used to measure the reaction mixture's absorbance at 415 nm following a 30-minute incubation period at room temperature. The same volume of distilled water was used as a blank in place of the 10% aluminum chloride. In a similar manner, the total flavonoid concentration was determined by reacting 0.5 ml of extracts with aluminum chloride.

***In vitro* anticancer screening**

Brine Shrimp Lethality Bioassay

Materials

***Artemia salina* eggs.** We purchased the eggs of brine shrimp (Artemia salina) from www.brineshrimpdirect.com. Small and brown in color, the eggs were tiny. About 2.5 to 3.0 thousand eggs are represented by 100 gram of eggs. The eggs were kept at room temperature in an opaque, airtight container. They can survive for many years under these favorable conditions.

Hatching chamber: In a beaker of seawater with continuous aeration, brine shrimp (Artemia salina Leach) eggs were hatched. The nauplii were harvested using a pipette on a lit background after 48 hours.

Method

Sample preparation. To create extract samples, 5 mg of extract was dissolved in 5 ml of DMSO to create a stock solution with 1000 µg/ml. To obtain the final drug concentration of 10 ppm, 50 ppm, and 100 ppm, 50 µl, 250 µl, and 500 µl were taken from this stock and their volume was increased to 5 ml (in a seven ml vial capacity) using a solution that contained a certain proportion of brine and yeast suspension. For every dose level, three dilutions were prepared. Equal amounts of distilled water were added to control vials.

Sea water. In accordance with the composition provided by Dr. Vasudevappa, Research Officer, FRS, Hesaragatta, Bangalore, sea water was prepared for brine shrimp hatching. In Table 1, the composition is provided. After being weighed appropriately, all major, minor, and trace salts were dissolved in 500 milliliters of new distilled water, and the volume was adjusted to five liters using distilled water. Before being used, it was filtered with Whatman filter paper.

Table 1: Composition of artificial sea water.

Sea Water Composition	
Constituents	**Quantity for 1000Lts**
Major Salts	
Sodium Chloride	11.82 kg
Magnesium Chloride	2.95kg
Magnesium Chloride	2.31kg
Calcium Chloride	0.59kg
Potassium Chloride	0.26kg
Sodium Bicarbonate	0.09kg
Minor salts	
Strontium Chloride	8.5 gm
Manganese sulphate	1.7gm
Sodium Phosphate	1.7gm
Lithium Chloride	0.43gm
Sodium Molybdate	0.43gm
Sodium Thiosulphate	0.43gm
Trace salts	
Potassium Bromide	11.5 gm
Aluminium Sulphate	0.37 gm
Zinc Sulphate	0.06 gm
Cobalt Sulphate	0.04 gm
Potassium Chloride	0.04 gm
Cupric Sulphate	0.04 gm

Bio Assay. Ten of these shrimp were moved to each sample vial after being counted in the pipette stem against a bright background. Nauplli were pulled in a pipette along with water. Five milliliters of fake seawater were added to each vial. As shrimp food, a drop of dry yeast suspension (3 mg in 5 ml sea water) was put to each vial. The vials were kept in an illuminated environment. Using a 3X magnifying lens, survivors were counted after 24 hours, and LD50 values and the proportion of deaths were computed.

***In vitro* cytotoxicity study by Tryphan Blue Exclusion assay**

Principle. Certain dyes can be kept from entering a living cell by blocking their passage through the membrane. As a result, under a light microscope, the living cells stay unstained and are easily separated from the dead cells, which absorb the dye and turn blue.

Cell Line. Ehrlich's Ascitic Carcinoma (EAC) cell lines were acquired from the Amala Cancer Research Center in Amala Nagar, Thrissur, Kerala, India, in order to cause cancer in mice. Through intraperitoneal inoculation, the cells were kept in Swiss albino mice as ascites tumors.

Preparation of Tryphan Blue solution: In 100ml of saline (0.9% NaCl), 100 mg of trypan blue was dissolved and stored at 4ºC.

Method· Following a PBS wash of the ascitic fluid extracted from the peritoneum of the EAC-inoculated animals, a hemocytometer was used to measure the cell viability using trypan blue dye. Under a microscope, the number of viable (unstained) and dead (stained) cells in each of the four corner squares of the WBC chamber were counted. The total number of viable cells per milliliter was then computed as follows:

Total number of cells = Mean number of cells × Dilution factor × 10^4

PBS was used to create the stock cell suspension for 1X107, and 0.1 ml of the solution was transferred to sterile test tubes. After treating the cells with 0.1 milliliters of extract solution at different doses (no more than 0.1% DMSO), 0.7 milliliters of PBS were added. For three hours, the cells were incubated at 370 C. Trypan blue (0.1 ml) was added and thoroughly blended after the exposure. A hemocytometer was used to measure the total number of living and dead cells in each of the four chamber corner squares, and the percentage viability/cytotoxicity was computed as follows:

Percent viable cells = (Number of unstained cells/Total number of cells) × 100.

***In vitro* cytotoxicity study in cultured cells by MTT assay**

MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] **assay:**

Principle. The "succinate tetrazolium reductase" system, which is a component of the mitochondrial respiratory chain that operates in metabolically active cells, absorbs MTT and converts it to formazan. Dimethyl sulfoxide (DMSO) is used to dissolve formazan, a purple water-insoluble substance that accumulates inside healthy cells due to its high impermeability to cell membranes. Using a standard ELISA plate reader, the optical density (OD) of the purple-colored solution that had formed was measured at 540 nm (maximum absorbance). It is possible to interpret the ability of cells to reduce MTT as a measure of viability and/or cell quantity since it indicates the integrity and activity of the mitochondria.

Materials

Cell line. Adenocarcinomic human alveolar basal epithelial cells (A-549), human breast adenocarcinoma cells (MCF-7), and human glioblastoma cells (U373) were purchased from NCCS (National Centre for Cell Science, Pune, India). were kept in media supplemented with 10% fetal bovine serum (FBS) and kept in Dulbecco's Minimum Essential Medium (DMEM). MTT reagent purchased from Sigma Aldrich in the United States. 96-well microculture plates and tissue culture flasks from Tarson and Nunc in the United States. Gentamycin from Manipal's KMC Hospital.

Maintenance of cell lines. Acquired from NCCS Pune, MCF-7, A549, and U373 were cultured in 25 cm^2 tissue culture flasks with DMEM media supplemented with 10% FBS, 1% L-glutamine, and 50µg/ml Gentamycin sulfate at 37ºC in a CO_2 incubator with humidified 5% CO_2 and 95% air. Regular subculturing was used to keep the cells alive in tissue culture flasks measuring 25 cm^3.

Sub culturing process of cell lines

— Sterile phosphate buffered saline (PBS) was used to wash the culture media after it was aspirated from the flasks holding the monolayer culture.

— Two milliliters of a 0.1% trypsin-EDTA solution were applied to each flask, aspirated a few seconds later, and then placed in an incubator for two to three minutes to allow for detachment.

— To separate all of the adherent cells, the flasks were taken out of the incubator and gently tapped. Using an inverted microscope (Nikon Eclipse TE 2000-5, Japan), the cell separation was verified.

— After the cells were fully separated from the flasks, two to three milliliters of the appropriate media containing 10% FBS were added and thoroughly mixed.
— Using a tiny sample of the suspension, the trypan blue dye exclusion test was used to determine the cell viability.
— A 25 cm^2 tissue culture flask containing around 4 ml of fresh media was seeded with 1 × 104 viable cells/ml suspended in media from the stock cell suspension. The flasks were then incubated until they reached 60–70% confluence.

Preservation of the tumor cells. In cryovials containing the same media supplemented with 10% FBS and 10% DMSO as a preservative, tumor cells from the first and second transplantation passages were kept in liquid nitrogen at a concentration of 1 × 10^6 cells/ml. The tumor bank was made up of this. Every ten passes, the original tumor cells from the tumor bank were used to start a new passage, discarding the tumor cell line.

Trypsinization. Trypsinization was used to remove the cells from the culture flasks in order to get a single cell suspension from a monolayer culture.
— A micropipette was used to aspirate the culture media out of a 60–70% confluent flask.
— Three milliliters of PBS were used to wash the cells in order to get rid of any remaining media.
— Two milliliters of trypsin-EDTA were introduced to each culture flask, aspirated a few seconds later, and then placed in the incubator for three to four minutes to allow the cells to separate.
— To make sure the cells were fully dislodged, culture flasks were examined using an inverted microscope (Nikon Eclipse, Japan).
— By adding two to three milliliters of medium containing 10% FBS, trypsin activity was inhibited.

MTT assay method. MCF-7, A549, and U373 melanoma cells that were developing exponentially were taken out of 25 cm^2 tissue culture flasks, and a stock cell solution (1X10^5cell/ml) was made.
— 1 × 10^4 cells were sown in 0.1 ml of DMEM media supplemented with 10% FBS in a 96-well flat bottom tissue culture plate, and the cells were left to adhere for 24 hours.
— Prior to the experiment, a stock solution of extracts was made in 0.5% DMSO and serially diluted with medium to provide working stock solutions of 50 µg/ml, 100 µg/ml, 250 µg/ml, and 500 µg/ml.
— Cells were treated with 20 µl of test solutions from the corresponding top stocks after 24 hours of incubation. Eighty µl of new media was then added, and the cells were incubated for 48 hours.
— Only the medium containing 0.5% DMSO was given to the cells in the control group.
— Every procedure was carried out in quadrate.
— Following the procedure, the drug-containing medium was taken out and cleaned with 200 µl of PBS.
— 100µl of MTT reagent (stock: 1 mg/ml in PBS) was applied to each well of the 96-well plate, and the plates were then incubated for 4 hours at 37ºC.
— To remove the MTT reagent, the plate was inverted over tissue paper after four hours of incubation.
— 100µl of 100% DMSO was applied to each well in order to dissolve the formazan crystals.
— An Enzyme Linked Immunosorbent Assay (ELISA) plate reader was used to assess the optical density (O.D.) at 540 nm.
— Each extract's percentage cytotoxicity was determined using the following formula:

$$\% \text{ Cytotoxicity} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

The results were plotted against the tested drug concentrations and shown as Mean ± SEM percentage O.D. values (proportional to cell survival).

The SRB assay. A suitable solvent that won't damage the cells was utilized to generate a stock solution of 40 mg/ml of aqueous and methanolic extract. Although dimethyl sulfoxide (DMSO) is frequently employed, it must be diluted with medium so that the final concentration used to treat the cells is less than 1% w/v because it is lethal at high doses. Using the SRB assay outlined below, tests should be conducted using any solvent to determine its cytotoxicity. Filter all stock solutions (0.22 lm pore size) to sterilize them, then store them at -200C.

Principle.SRB is an aminoxanthane dye that is brilliant pink and contains two sulphonic groups. SRB provides a sensitive indicator of cellular protein content that is linear over a cell density of at least two orders of magnitude when it binds to protein basic amino acid residues in trichloroacetic acid-fixed cells under mildly acidic circumstances.
In SRB test, color development happens quickly, steadily, and visibly. A spectrophotometer or a 96-well plate reader can be used to measure the developed color over a wide range of visible wavelengths.

Procedure. Added 100 µl of optimally dense cell suspension to each 96-well plate well.
1. To ensure that, after being diluted twice, the final concentrations in the wells ranged from 100 to 0.2 µg/ml, a range of concentrations of the compounds to be evaluated were created in the culture medium.
2. Fill the cell-containing wells with 100 µl of each test sample concentration in culture media. Only the control wells received the addition of 100 µl of media.
3. The cells and samples were incubated for 48 hours.
4. For one hour at 40 degrees Celsius, cells were fixed with ice-cold Trichloroacetic acid.
5. After five rounds of washing in distilled water, the plates were left to air dry.
6. Staining was allowed for 30 minutes at room temperature after 50 µl of sulphorhodamine (SRB) solution was added to each well of the dry 96-well plates.
7. To get rid of the unbound dye, the plates were rapidly cleaned five times with 1% v/v acetic acid to remove the sulphorhodamine (SRB) solution.
8. Air dried the cleaned plates. By adding 100 µl of 10 mM unbuffered Tris Base (pH 10.5) to each well and shaking for five minutes on a shaker platform, the bound SRB was dissolved.
9. A 96-well plate reader operating at 492 nm was used to read the plates.

10. The OD values were plotted versus concentration in order to establish the IC50 because the optical density (OD) of SRB in each well was directly proportional to the number of cells.

SRB recovery assay. As with the exposure assay above, steps 1–4 were carried out.

1. After five rounds of washing in distilled water, the plates were left to air dry.
2. Each well received 200 µl of medium.
3. Incubated for seventy-two hours.
4. Completed the SRB assay in accordance with the aforementioned steps 5–11.

Cell lines and normal culture conditions. NCCS (National Centre for Cell Science, Pune) provided the MCF7, U343, and A549 cell lines. The cells were cultured in an incubator at 37°C with 5% CO^2 in DMEM (Dulbecco's Minimum Essential Medium) media supplemented with 10% fetal bovine serum (FBS) and Sigma Aldrich's MTT reagent. 96-well Tarson and Nunc microculture plates and tissue culture flasks.

RESULTS

Physicochemical constants

Ash values

Table 2: Ash values.

Parameters	Value
Total ash	7.83 %w/w
Acid insoluble ash	2.44 %w/w
Water soluble ash	5.49 %w/w
Sulphated ash	5.85 %w/w

Extractive values

Table 3: Extractive values.

Parameters	Value
Water soluble extractive	15.7 %w/w
Ethanol soluble extractive	7.6 %w/w
Ether soluble extractive	2.2%w/w

Moisture content. Loss on drying at 105ºC: 8.76 %w/w

Foaming index. Foaming index of stem powder: NIL

Tannin content. Tannin content of stem powder: 3.0 % w/w

Swelling index. Swelling index of stem powder: 2

Phytochemical study

Preliminary phytochemical screening

Table 4: Successive solvent extraction.

Sr. No.	Solvent	Color	Weight of the extract (g)
1.	Petroleum ether (60-80°C)	Dull green	2.5
2.	Benzene	Dark greenish brown	2.2
3.	Chloroform	Dull brown	2.9
4.	Acetone	Dirty brown	3.4
5.	Ethanol (95%)	Blackish brown	5.3
6.	Chloroform: Water (1:99)	Brown	5.8

Phytochemical analysis of different extracts (Qualitative chemical tests)

Table 5: Qualitative chemical tests of successive extracts.

Test	Petroleum ether	Benzene	Chloroform	Acetone	Ethanol	Water
Alkaloids	-	-	-	-	-	+
Carbohydrates	+	-	-	-	+	+
Phytosterols	+	-	-	+	+	-
Fixed oils and fats	+	+	-	-	+	-
Saponins	-	-	-	-	-	-
Phenolic compounds and tannins	-	-	-	-	+	+
Proteins	-	+	-	-	+	+
Gums and mucilages	-	-	-	-	-	-
Flavonoids	-	-	+	+	+	+

(+ Present, - Absent)

Total Phenolic content. When compared to gallic acid, the total phenolic content of the methanolic and aqueous extracts of M. parasiticus stem was 1.25 and 0.68 mg/g, respectively.

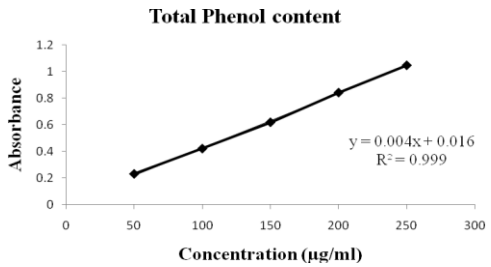

Fig. 1. Standard plot of Gallic acid.

Total Flavonoid content. When compared to rutin, the total flavonoid concentration of the methanolic and aqueous extracts of M. parasiticus stem was 0.52 mg/g and 0.78 mg/g, respectively.

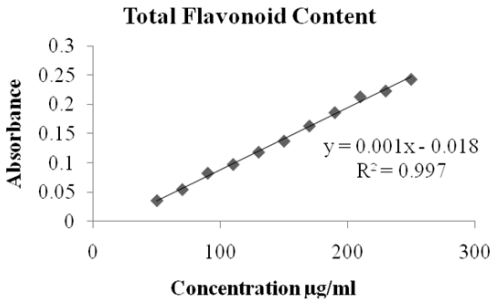

Fig. 2. Standard plot of rutin.

Table 6: LC_{50} value of various extracts on brine shrimp lethality bioassay.

Extract	Mean % death after 24 hrs					LC_{50} (µg/ml)
	62.5 (µg/ml)	125(µg/ml)	250 (µg/ml)	500 (µg/ml)	1000 (µg/ml)	
Control	0.00	0.00	0.00	0.00	0.00	-
Ethanolic extract	26.00 ± 0.57**	54.00 ± 1.73**	69.66 ± 1.85**	79.22 ± 0.88**	88.24 ± 1.44**	
Aqueous extract	34.00 ± 0.12**	62.67 ±1.97**	75.32 ± 0.76**	87.32 ± 1.67**	96.56 ± 2.32**	90

*****All the values are mean±SEM of three samples, **P < 0.01 compared to control

Table 7: *In-vitro* cytotoxic effect of various extracts on EAC cells by tryphan blue exclusion assay.

Extracts	Percentage death after 3hrs.		
	10 µg/ml	50 µg/ml	100 µg/ml
Control	3.84±0.14	3.84±0.14	3.84±0.14
Ethanolic extract	6.56±0.85	24.74±1.28	49.44±1.43
Aqueous extract	9.62±1.49*	31.31±0.93*	56.29±1.70*

*All the values are mean ± SEM of three samples, *P < 0.05 compared to control.

Table 8: *In vitro* cytotoxic activity of various extracts in MCF-7 cells (Human breast adenocarcinoma cells) by MTT assay at 48 hours of exposure.

Extracts	Percentage Cytotoxicity (48 hrs.)			
	62.5 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml
Control	0	0	0	0
Ethanolic extract	30.02±4.63**	57.41±1.19**	78.55±2.73**	91.79±2.31**
Aqueous extract	39.33±1.28**	64.53±0.22**	88.07±0.28**	97.79±1.06**

*****All the values are mean ± SEM of three samples, **P < 0.01 compared to control

Table 9: *In vitro* cytotoxic activity of various extracts in U343 cells (Human neuronal glioblastoma cells) by MTT assay at 48 hours of exposure.

Extracts	Percentage Cytotoxicity (48 hrs.)			
	62.5µg/ml	125µg/ml	250µg/ml	500µg/ml
Control	0	0	0	0
Ethanolic extract	27.20±2.79**	55.54±1.91**	76.43±2.13**	89.21±1.71**
Aqueous extract	35.38±0.87**	63.19±0.42**	82.62±1.56**	96.34±1.20**

*****All the values are mean ± SEM of three samples, **P < 0.01 compared to control

Table 10: *In vitro* cytotoxic activity of various extracts in A549 cells (Human lung adrenocarcinoma cells) by MTT assay at 48 hours of exposure.

Extracts	Percentage Cytotoxicity (48 hrs.)			
	62.5µg/ml	125µg/ml	250µg/ml	500µg/ml
Control	0	0	0	0
Ethanolic extract	25.55±1.79**	51.54±1.41**	67.70±2.60**	81.11±1.67**
Aqueous extract	30.83±0.68**	59.78±1.24**	73.62±1.76**	89.14±1.26**

*****All the values are mean±SEM of three samples, **P < 0.01 compared to control

Table 11: *In vitro* cytotoxic activity of various extracts in MCF-7 cells (Human breast adenocarcinoma cells) by SRB assay at 48 hours of exposure.

Extracts	Percentage Cytotoxicity (48 hrs.)			
	62.5µg/ml	125µg/ml	250µg/ml	500µg/ml
Control	0	0	0	0
Ethanolic extract	28.02±2.63**	55.41±1.16**	72.55±2.39**	87.79±1.31**
Aqueous extract	34.33±1.96**	62.53±0.22**	81.07±1.28**	92.79±0.16**

*****All the values are mean±SEM of three samples, **P < 0.01 compared to control

Table 12: *In vitro* cytotoxic activity of various extracts in U343 cells (Human neuronal glioblastoma cells) by SRB assay at 48 hours of exposure:

Extracts	Percentage Cytotoxicity (48 hrs.)			
	62.5µg/ml	125µg/ml	250µg/ml	500µg/ml
Control	0	0	0	0
Ethanolic extract	26.62±1.91**	53.38±2.13**	67.21±1.56**	82.34±1.20**
Aqueous extract	30.83±1.44**	56.97±1.73**	73.79±0.87**	88.54±0.42**

*****All the values are mean±SEM of three samples, **P < 0.01 compared to control

Table 13: *In vitro* cytotoxic activity of various extracts in A549 cells (Human lung adrenocarcinoma cells) by SRB assay at 48 hours of exposure.

Extracts	Percentage Cytotoxicity (48 hrs.)			
	62.5µg/ml	125µg/ml	250µg/ml	500µg/ml
Control	0	0	0	0
Ethanolic extract	24.46±1.14*	50.97±2.10*	62.34±1.86*	76.81±2.21*
Aqueous extract	28.29±1.26*	54.85±0.96*	68.27±1.31*	82.16±1.89**

*****All the values are mean±SEM of three samples,*P < 0.05, **P < 0.01, ***P < 0.0001 compared to control

CONCLUSIONS

Cancer remains a significant global health challenge, necessitating the search for new and effective therapeutic agents. Medicinal plants, such as *Macrosolen parasiticus*, offer a promising avenue for discovering novel anticancer compounds due to their rich phytochemical diversity and traditional medicinal use. This study aims to evaluate the in vitro anticancer potential of *Macrosolen parasiticus* stem extracts, focusing on its cytotoxic effects, apoptosis induction, and possible mechanisms of action. The results of this research could contribute to the growing field of plant-based anticancer therapies and provide valuable insights for future drug development.

FUTURE SCOPE

The future scope of evaluating the in vitro anti-cancer potential of Macrosolen parasiticus stem is vast and requires further exploration to establish its efficacy and therapeutic potential. Future studies should focus on *in vivo* investigations using animal models to validate its anticancer activity, bioavailability, and metabolism. Additionally, the isolation and characterization of bioactive compounds through advanced techniques like HPLC, LC-MS, and NMR will help identify the key phytochemicals responsible for its anticancer effects.

Acknowledgement. The author is thankful to Department of Pharmaceutical Sciences, Apex University, Jaipur for providing facilities.
Conflict of Interest. None.

REFERENCES

Ahmed, S. & Hasan, M. M. (2015). Standardization of crude drugs: A precise review. *World J Pharm Res*, *4*(10), 155-74.

Bunn, C. W., Clark, L. M. & Clifford, I. L. (1935). The constitution and formation of bleaching powder. Proceedings of the Royal Society of London. *Series A-Mathematical and Physical Sciences*, *151*(872), 141-167.

Camp, D., Davis, R. A., Campitelli, M., Ebdon, J. & Quinn, R. J. (2012). Drug-like properties: guiding principles for the design of natural product libraries. *Journal of natural products*, *75*(1), 72-81.

Etiegni, L. & Campbell, A. G. (1991). Physical and chemical characteristics of wood ash. *Bioresource technology*, *37*(2), 173-178.

Galati, G. & O'brien, P. J. (2004). Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. *Free radical biology and medicine*, *37*(3), 287-303.

Gul, S. & Safdar, M. (2009). Proximate composition and mineral analysis of cinnamon. *Pakistan Journal of Nutrition*, *8*(9), 1456-1460.

Himesh, S., Sharan, P. S., Mishra, K., Govind, N. & Singhai, A. K. (2011). Qualitative and quantitative profile of curcumin from ethanolic extract of Curcuma longa. *Int Res J Pharm*, *2*(4), 180-184.

Houck, K. A. & Kavlock, R. J. (2008). Understanding mechanisms of toxicity: insights from drug discovery research. *Toxicology and applied pharmacology*, *227*(2), 163-178.

Kumar, S. A., Manikgantan, E. & Ravichandran, M. (2023). In Vitro Evaluation of Anti Cancer and Antioxidant Activities of Siddha Polyherbal Formulation – Maramanjal Chooranam. *Biological Forum – An International Journal*, *15*(3a), 34-39.

Lux, A., Morita, S., Abe, J. U. N. & Ito, K. (2005). An improved method for clearing and staining free-hand sections and whole-mount samples. *Annals of Botany*, *96*(6), 989-996.

Mareel, M. & Leroy, A. (2003). Clinical, cellular, and molecular aspects of cancer invasion. *Physiological reviews*, *83*(2), 337-376.

Michalek, J. J. & Papalambros, P. Y. (2005). An efficient weighting update method to achieve acceptable consistency deviation in analytical target cascading. *J. Mech. Des.*, *127*(2), 206-214.

Muller, N., Girard, P., Hacker, D. L., Jordan, M. & Wurm, F. M. (2005). Orbital shaker technology for the cultivation of mammalian cells in suspension. *Biotechnology and Bioengineering*, *89*(4), 400-406.

Neat, F. U. & Berk, A. A. (1954). Analysis of Water-Formed Deposits. *Industrial & Engineering Chemistry*, *46*(5), 961-970.

Ng, S. B., Yan, J., Huang, G., Selvarajan, V., Tay, J. L. S., Lin, B. & Chng, W. J. (2011). Dysregulated microRNAs affect pathways and targets of biologic relevance in nasal-type natural killer/T-cell lymphoma. Blood. *The Journal of the American Society of Hematology*, *118*(18), 4919-4929

Patil, B. S., Jayaprakasha, G. K., Chidambara Murthy, K. N. & Vikram, A. (2009). Bioactive compounds: historical perspectives, opportunities, and challenges. *Journal of agricultural and food chemistry*, *57*(18), 8142-8160.

Pooja, M., Shamsiya, L., Suresh, S. & Balagurusamy, K. (2023). In-Vitro Cytotoxicity of Gandhaga Rasayanam (GR) on Human Breast Cancer (MCF-7) Cells. *Biological Forum – An International Journal*, *15*(3a), 46-54.

Sharath, K. P. & Naika, R. (2022). Gas chromatography-Mass spectroscopy analysis of Bioactive compounds from Methanolic leaf Extract of *Macrosolen parasiticus* (L.) Danser. *Indian J. Applied & Pure Bio.*, *37*(2), 524-530.

Shukla, S. & Mehta, A. (2015). Anticancer potential of medicinal plants and their phytochemicals: a review. *Brazilian Journal of Botany, 38,* 199-210.

Těšitel, J., Plavcová, L. & Cameron, D. D. (2010). Interactions between hemiparasitic plants and their hosts: the importance of organic carbon transfer. *Plant Signaling & Behavior, 5*(9), 1072-1076.

Whitehill, J. G., Henderson, H., Schuetz, M., Skyba, O., Yuen, M. M. S., King, J. & Bohlmann, J. (2016). Histology and cell wall biochemistry of stone cells in the physical defence of conifers against insects. *Plant, Cell & Environment, 39*(8), 1646-1661.

How to cite this article: Rekha Kanwar (2023). Evaluation of *in vitro* Anti-cancer Potential of *Macrosolen parasiticus* Stem. *Biological Forum – An International Journal, 15*(6): 1020-1029.