Research Article

Preliminary Phytochemical Analysis and Cytotoxicity Potential of *Bacopa monnieri* on Oral Cancer Cell Lines

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ABSTRACT

Medicinal plants have been identified and used throughout human history. In India, the Ayurveda medicinal system is based on herbs. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend the body. Ethnobotany, the study of traditional human uses of plants, is recognized as an effective way to discover future medicines. Some plants contain phytochemicals that have positive effects on the body. Chemical compounds in plants mediate their effect on the human body through processes identical to conventional drugs. This enables herbal medicines to have beneficial pharmacology.

Keywords: *Bacopa monnieri*, Medicinal plant, Phytochemical Analysis, Cytotoxicity.

INTRODUCTION

Medicinal plants have been identified and used throughout human history. In India, the Ayurveda medicinal system is based on herbs. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend the body. Ethnobotany, the study of traditional human uses of plants, is recognized as an effective way to discover future medicines. Some plants contain phytochemicals that have positive effects on the body. Chemical compounds in plants mediate their effect on the human body through processes identical to conventional drugs. This enables herbal medicines to have beneficial pharmacology.

One such medicinal herb used in Ayurveda as "Brahmi"[1], is *Bacopa monnieri* (waterhyssop, thyme-leafed gratiola, water hyssop, herb of grace,[1] Indian pennywort[1]),which is a perennial, creeping herb native to the wetlands of southern India, Australia, Europe, Africa, Asia, North and South America.[1]

Bacopa monnieri is an antioxidant[2]. *Bacopa monnieri* displays *in vitro* antioxidant and cell-protective effects[3]. *Bacopa monnieri* interacts with the dopamine and serotonergic systems, but its main mechanism concerns promoting neuron communication. It does this by enhancing the rate at which the nervous system can communicate by increasing the growth of nerve endings, also called dendrites.

In animals, it also inhibits acetylcholinesterase, activates choline acetyltransferase, and increases cerebral blood flow[4]. Several studies have suggested that *Bacopa monnieri* extracts may have protective effects in animal models of neurodegeneration.[5] Aqueous extracts of *Bacopa monnieri* may have reversible adverse effects on spermatogenesis, sperm count, and fertility in male mice.[6] The most commonly reported adverse side effects of *Bacopa monnieri* in humans are nausea, increased intestinal motility, and gastrointestinal upset.[7,8]

One of the diseases that dominate today's society is cancer, which is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body.[9] There are over 100 different known cancers that affect humans.[10] Cancer is often treated with some combination of radiation therapy, surgery, chemotherapy, and targeted therapy.[9,11]

However an emerging prospect for treatment of cancer is the use of herbs like *Bacopa mooneira* due to their phytochemical and cytotoxic properties against cancer. Organic extracts of B9_Pink (*Fusarium oxysporum*) and B19 (Fomitopsis sp.) are found to posses potent cytotoxic and antimicrobial properties, highlighting their possible potential for use in the development of anti-cancer drugs, which needs to be further studied[12].

The "phyto-" of the word phytochemicals is derived from the Greek word 'Phyto', which means plant. Therefore, phytochemicals are plant chemicals. Phytochemicals are defined as bioactive non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods that have been linked to reducing the risk of major chronic diseases[13].

Phytochemicals have the potential to stimulate the immune system, block substances we eat, drink and breathe from becoming carcinogens, reduce the kind of inflammation that makes cancer growth more likely, prevent DNA damage and help with DNA repair, reduce

the kind of oxidative damage to cells that can spark cancer, slow the growth rate of cancer cells, trigger damaged cells to commit suicide before they can reproduce and help to regulate hormones thereby resisting the risk of cancer.

Thousands of phytochemicals have been identified so far, and scientists have only begun to investigate their promise. Natural dietary phytochemicals have been widely used *in vitro*, *in vivo*, and preclinical cancer prevention and treatment studies.

Some of these clinical trials have shown various degrees of success[14].

Through extensive mechanistic studies, a robust chemo preventive effect of the phytochemicals[14] was observed.

Cytotoxicity is the degree to which an agent has specific destructive action on certain cells.

It is the possession of destructive action, particularly in reference to lyses of cells by immune phenomena.

Cell proliferation rates or viability levels are good indicators of cell health. Proliferation or viability analysis which is crucial for cell growth and differentiation studies, and are often coupled with metabolism analysis.

Assessing compound cytotoxicity is also a critical step in pharmaceutical development.

These assays in oncological settings are also used to evaluate both compound toxicity and inhibition of tumor cell growth during drug development.

MATERIALS AND METHODS

Preparation of plant extract

Bacopa monnieri contains active constituents like glycosides and hence a polar solvent like aqueous ethanol is used. The samples of plant (air dried and powdered), was commercially bought.

The dried powder was cold extracted with 50% ethanol for seven days with occasional stirring. The process was repeated twice.

The pooled extracts were concentrated, evaporated to dryness under reduced pressure.

The extract was re-suspended in 1% gum acacia and subjected to the various studies. All the chemicals used in the present study is of analytical reagent quality.

Maintenance of cell line

The vial containing the KB cell lines acquired from ATCC (CCL -17) was removed from liquid nitrogen freezer and immediately placed in a 37°C water bath. It was agitated continuously until the medium thawed.

Then it was centrifuged for 10 min at 150 to 200 × g, room temperature. Supernatant was discarded and cells were washed with fresh medium to remove residual DMSO.

The cell pellet was re-suspended in 3ml of DMEM with 10% FBS. It was then incubated in a CO2 incubator at a humidified 37°C. The medium was changed every 2 to 3 days or when pH indicator (e.g. phenol red) in medium changed colour.

The culture was kept in a medium with 10% FBS until cell line were re-established.

Phytochemical Tests

Test for carbohydrates

To 2ml of the plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates[15].

Test for tannins

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins[16].

Test for saponins

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins[17,18].

Test for flavonoids

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids[19].

Test for cardiac glycosides

To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added.

This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicates presence of cardiac glycosides[19].

Test for Terpenoids

To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates presence of terpenoids[19].

Phlobatannins

To 1ml of plant extract few drops of 2% HCL was added appearance of red color precipitate indicates the presence of Phlobatannins[19].

Anthraquinones

To 1ml of plant extract a few drops of 10% ammonia solution was added, appearance of a pink color precipitate indicates the presence of Anthraquinones[19].

Test for alkaloids

To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were

added. Presence of green color or white precipitate indicates the presence of alkal oids[20].

Test for quinones

To 1ml of extract, 1ml of concentrated Sulphuric acid was added.

Formation of red color indicates presence of quinones[21].

Test for phenols

To 1ml of the extract, a few drops of Phenol Ciocalteau reagent was added followed by few drops of 15% Sodium carbonate solution.

Formation of blue or green color indicates presence of phenols[21].

Test for coumarins

To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates presence of coumarins[21].

Test for glycosides

To 2ml of plant extract, 3ml of chloroform and 10% ammonia solution was added.

Formation of pink color indicates presence of glycosides[22].

Steroids and phytosteroids

To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids[23].

MTT assay for cell viability

The MTT assay (Mossman, 1983)[24] is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product.

Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at 37°C in humidified atmosphere with 5% CO_2.

The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2X 10^4 cells/well and allowed to attach overnight at 37°C.

The medium was then discarded and cells incubated with different concentrations of the samples (25, 50, 75,100& 125µL) for 24 hours.

After the incubation, medium was discarded and 100µl fresh medium was added with 10µl of MTT (5mg/ml). After 4 hours, the medium was discarded and 100µl of DMSO was added to dissolve the formazan crystals.

Then, the absorbance was read at 570nm in a microtitre plate reader. Cyclophosphamide was used as a positive control.

Cell survival was calculated by the following formula:

Viability % = (Test OD/ Control OD) X 100

Cytotoxicity % = 100 – Viability%

RESULTS AND DISCUSSION

Phytochemical Tests

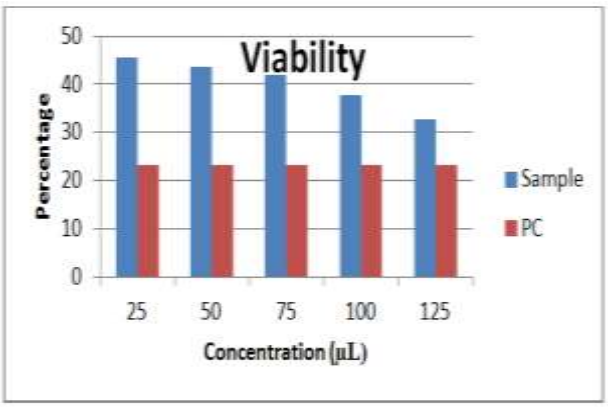
1	Carbohydrates test	+
2	Tannins test	+
3	Saponins test	-
4	Flavonoids test	Weakly +
5	Alkaloid test	+
6	Quinones test	+
7	Glycosides test	Weakly-
8	Cardiac glycosides test	+
9	Terpenoids test	-
10	Phenols test	+
11	Coumarins test	Weakly +
12	Steroids & Phytosteroids	-
13	Phlobatannins test	-
14	Anthraquinones test	-

MTT assay

Table 1: Percentage of cell viability of Sample and positive control against KB cells

	Viability in percentage	
Concentration (µL)	**Sample**	**PC**
25	45.63	23.1
50	43.58	23.1
75	41.84	23.1
100	37.63	23.1
125	32.82	23.1

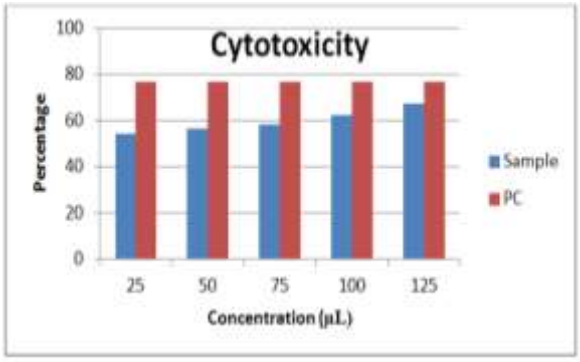
PC-Positive control (Cyclophosphamide), C-Control



Graph 1

Table 2: Shows percentage of cytotoxicity of Sample and positive control against KB cells

	Cytotoxicity in percentage	
Concentration (µL)	**Sample**	**PC**
25	54.37	76.9
50	56.42	76.9
75	58.16	76.9
100	62.37	76.9
125	67.18	76.9



Graph 2: Effect of cytotoxicity of sample and positive control in KB cells

PC-Positive control (Cyclophosphamide)

The main constituents found in the extract were carbohydrates, tannins, alkaloids, quinones, cardiac glycosides and phenols. The minor constituents include flavinoids and coumarins. Secondary metabolites such as alkaloids, quinones and phenols, present in *Bacopa monnieri* showed anticancer potential. The presence of phenols suggests the antioxidant activity of the herb[25]. Tannins which are a group of phenolic compounds that are known antimutagentic property and can act against cancer cells[26]. Cytotoxicity analysis by using varying concentration of *Bacopa monnieri* extract (25, 50, 75, 100& 125µL) was done. As seen in Table 1 the viability of the KB cell lines show a gradual decrease as the concentration of the extract is increased. In Table 2, *Bacopa monnieri* exhibited increasing cytotoxicity with increasing concentration. This is also evident from the visual representation of the same data in graph 1 and graph 2.

CONCLUSION

The use of natural products for treatment is generally a more preferred option due to the adverse side effects which accompany other treatment plans. A phytochemical analysis was also done.

The main constituents found in the plant extract were alkaloids, quinones, phenols and tannins.

The presence of these secondary metabolites reveals the antioxidant property of the extract. The study exposed the cytotoxic potential and antitumor properties of *Bacopa monnieri*, a medicinal herb.

The plant extract presented cytotoxic effect in high concentrations, leading to increased cell death in the KB cell culture. The potential to exploit *Bacopa monnieri* as an anticancer drug is a thrust area for future research.

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