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Research Article

ANTICANCER POTENTIAL OF *BAMBUSA BAMBOS* LEAF EXTRACTS

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ABSTRACT
Anti-cancer activities of chloroform and hydro-alcohol leaf extracts of *Bambusa bambos* was evaluated in vitro using Dalton's Lymphoma Ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) cell lines by Trypan blue dye exclusion method. The chloroform extract exhibited better activity compared to hydro-alcohol extract. Further hemolytic activities of both the extracts were carried out to measure the extent of damage to normal red blood cell membranes. The findings suggested that both the extracts produced no signs of hemolysis indicating that the extracts are not toxic to normal erythrocytes.
Key words: *Bambusa bambos*, Cytotoxicity, Hemolytic, Poaceae.

INTRODUCTION

Cancer is a general term for uncontrolled growth of abnormal cells medically known as neoplasm characterized by autonomous growth of tissues and loss of differentiation. Tumour mass may metastasis and spread to other tissues and organs[1]. Cancer is one of the leading causes for mortality worldwide and the inadequacy of conventional chemotherapy to effect major reduction in mortality indicates that new approaches are critically needed. It is estimated that 7.6 million people died of cancer in 2007 worldwide and it is projected to increase to 11.5 million deaths by 2030 [2]. The low efficacy of current chemotherapy accompanied with severe adverse reactions has been driving an increasing number of patients toward alternative medicine. In the United States, half of all patients with cancer have tried complementary and alternative medicine [3]. Therefore, there is an urgent need to develop new anticancer agents with minimum side effects. From the earliest times, herbs have been prized for their pain-relieving and healing abilities and today we still rely largely on the curative properties of plants. According to World Health Organization, 80% of the people living in rural areas depend on medicinal herbs as primary healthcare system [4]. The synthetic anticancer remedies are beyond the reach of common man because of cost factor. Herbal medicines have a vital role in the prevention and treatment of cancer and medicinal herbs are commonly available and comparatively economical [4]. Of the 92 anticancer drugs approved between 1983 and 1994 for commercial use approximately 62% are directly related to natural origin. Plant derived natural products such as flavonoids, terpenes, alkaloids[5] etc. have received considerable attention in recent years due to their diverse pharmacological properties including anti-oxidant and cancer chemo-preventive effects [6]. Free radical damage may lead to cancer. Some of the natural products are rich antioxidants. Antioxidants interact with these radicals and may prevent the damage caused by them.

Bambusa bambos (*Poaceae*), known as Bamboo (English) are a group of woody perennial grasses found throughout India and in tropical and subtropical areas, especially in the monsoon and wet tropics. Chemical constituents includes Silica 90%, potash, lime, alumina, cholin, betain, hydrate of silicic acid, nuclease, urease, proteolytic enzyme, cyanogentic glucoside and an alkaloid [7]. The main parts used are leaves, shoots, seeds, fruits, and manna. The leaves and stems of *Bambusa bambos* have been used in Indian folk medicine to treat inflammatory conditions, as coolant, laxative, wounds and piles. The seeds are used as aphrodisiac, in biliousness and urinary discharges. Other traditional uses are astringent, emmanogogue, and febrifuge to heal the wounds and also to control diarrhea in cattle [7]. Manna (crystalline substance found inside the bamboo) and leaves are used in Ayurvedic medicine in ptosis and paralytic complaints [8]. Tabasheer, a siliceous secretion (up to 97% SiO_2), considered aphrodisiac, cooling, and tonic, is used in asthma, cough and debilitating diseases [9]. Though the plant and its extracts have been extensively used in the folklore medicine, information from organized search of published literature does not provide evidence for its antitumor activities. Also the increase in the use of medicinal plants and their phyto-constituents in recent times, as well as the scarcity of scientific studies on their safety and efficacy have raised concerns in the scientific community and there is a need to assess the potential effects of these plants. Keeping this in view, the present study has been undertaken to investigate the anticancer potential of extract of *Bambusa bambos.*

MATERIALS AND METHODS

Collection of Plant material

The leaves of *Bambusa bambos* were collected from Palode Botanical Garden, Trivandrum during the month of October. The plant material was identified by the Botanist DR. K. C. Koshy, Scientist E-1, Tropical Botanical Garden Research Instituite, Palode.

Preparation of leaf extracts

The leaves were dried under the shade and then powdered with mechanical grinder and stored in an air tight container for the preparation of chloroform and hydro-alcohol extracts. The powdered leaves were extracted with chloroform by Soxhlet extraction method. Hydro-alcohol extract was prepared by taking 140 grams of powdered leaves of

Bambusa bambos in 2.5-litre capacity glass container and adding 300ml ethanol and 700ml water. The mixture was shaken occasionally for 3-4 days. It was then filtered, solvents were evaporated and the extract was stored in a desiccator till when needed.

Ash values

The ash value is an important parameter for the evaluation of crude drugs, due to the variation of values within fairly wide limits .The ash value of any organic material is composed of inorganic materials like metallic salts and silica. Ashing involves an oxidation of the components of the products; a high ash value involves the contamination, substitution, adulteration or carelessness in the preparation of crude drugs for marketing.

Total ash: 4g of the ground air dried material was accurately weighed and placed in a previously ignited and weighed crucible. The material was spread in an even layer and was ignited at a temperature of 500-600°C until the residue turned white, indicating the absence of carbon. The crucible was cooled in a desiccator and weighed for constant weight. The content of total ash per gram of air dried material was then calculated. All the values are taken in triplicate and their mean values are calculated.

Acid insoluble ash: To the crucible containing the total ash, 25 ml of Hydrochloric acid was added, covered with a watch-glass and heated gently for 5 minutes. The watch-glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The contents were filtered and the insoluble matter was collected on an ashless filter-paper which was washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matter was then transferred to the original crucible dried on a hot plate and ignited to a constant weight and allowed to cool for 30 minutes and then reweighed. The content of acid-insoluble ash per gram of air-dried material was then calculated. All the values are taken in triplicate and their mean values are calculated.

Water soluble ash: To the crucible containing the total ash, 25 ml of water was added and heated for 5 minutes. The insoluble matter was collected on an ashless filter-paper, washed with hot water, transferred to the crucible then and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of this residue was subtracted from the weight of total ash and the content of water-soluble ash per gram of air-dried material was then calculated. All the values are taken in triplicate and their mean values are calculated.

Cytotoxicity studies

The extracts at toxic concentration damage the cells and makes pores on the membrane through which trypan blue enters. The damaged cells are stained with trypan blue stain and can be distinguished from viable cells. Since live cells are excluded from staining, this method is also known as dye exclusion method [10].

Erhlich's Ascites Carcinoma (EAC): Varying concentrations (10 - 200µg/ml) of chloroform and hydro-alcohol extracts of leaves of *Bambusa bambos* were prepared. The cancer cells were aspirated from the peritoneal cavity of cancer bearing mice and were washed thrice with normal saline. The cell suspension (1 x 10^6 EAC cells in 0.1 ml) was added to tubes containing various concentration of test extracts (10, 20, 50,100 and 200 µg/ml) and volume was made up to 1ml using phosphate buffer saline (PBS). The control tube contained only EAC cell suspension. The assay mixtures were incubated for 3 hours at 37^0C and were then added with two drops of Trypan blue dye. Further % of dead cells was evaluated by Trypan Blue Exclusion method.

% Cytotoxicity = No. of dead cells / (No. of live cells+ No. of dead cells) x 100

Dalton's Lymphoma Ascites (DLA): Varying concentrations (10 - 200µg/ml) of chloroform and hydro-alcohol extracts of leaves of *Bambusa bambos* were prepared. The cancer cells were aspirated from the peritoneal cavity of cancer bearing mice and were washed thrice with normal saline. The cell suspensions (1x 10^6 DLA cells in 0.1 ml) was added to tubes containing various concentration of test extracts (10, 20, 50,100 and 200 µg/ml) and volume was made up to 1ml using phosphate buffer saline (PBS). Control tube contained only cell suspension. The assay mixtures were incubated for 3 hours at 37 ^{0}C and % of dead cells were evaluated by Trypan Blue Exclusion method.

% Cytotoxicity = No. of dead cells / (No. of live cells+ No. of dead cells) x 100

Hemolytic activity

Hemolysis is the rupturing of erythrocytes and the release of their contents (hemoglobin) into surrounding fluid (*e.g.* blood plasma). Hemolysis may occur in vivo or.The hemolytic activity rapidly measures the extent of damage to red blood cell membranes. Release of hemoglobin into the extracellular solution makes the solution red in in vitro appearance. In the present study hemolytic activity was performed to confirm the safety of the test extracts on normal cell membrane. Different concentrations of the leaf extracts were prepared in phosphate buffer pH 7.4 in four test tubes and were added with a constant volume of blood suspension (2%v/v in phosphate buffer) totally to make the final volume for two ml. The test tubes were gently inverted in order to mix the contents. The tubes were then shaken after 30 minutes and were allowed to stand for 6 hours at room temperature. The tubes were examined for any signs of hemolysis, indicated by reddish brown coloured solution without any deposits of erythrocytes. The same above procedure was followed by taking Mercuric chloride as standard in the absence of the test extracts and the test tubes were observed for the signs of hemolysis.

RESULTS & DISCUSSION

Ash values

The leaf extracts of the plant *Bambusa bambos* were subjected for the determination of various ash values. The total ash value was found to be 94.62 mg/gm, acid insoluble ash values were found to be 48.24 mg/gm and the water soluble ash value was found to be 19.81 mg/gm (Figure 1).

Table 1: Cytotoxic activity of Chloroform and hydro-alcohol extracts of *Bambusa bambos* in Erlich's Ascites Carcinoma cell lines

Concentration extract (µg/ml)	% Cytotoxicity (EAC)	
	Chloroform extract	Hydro –alcohol extract
10	21	12
20	45	15
50	70	31
100	75	49
200	80	60

Table 2: Cytotoxic activity of Chloroform and hydro-alcohol extracts of *Bambusa bambos* in Dalton's Lymphoma Ascites cell lines

Concentration extract (µg/ml)	% Cytotoxicity (DLA)	
	Chloroform extract	Hydro –alcohol extract
10	32	15
20	42	17
50	63	25
100	86	29
200	100	48

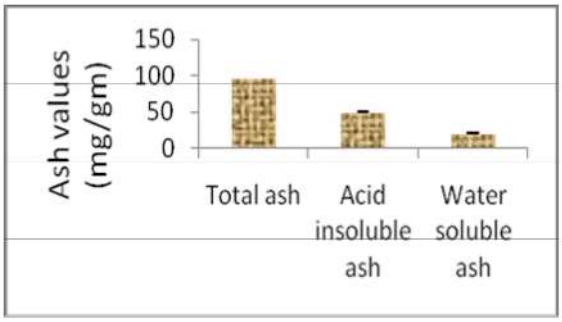


Figure 1: Ash values of the leaves of the plant *Bambusa bambos*

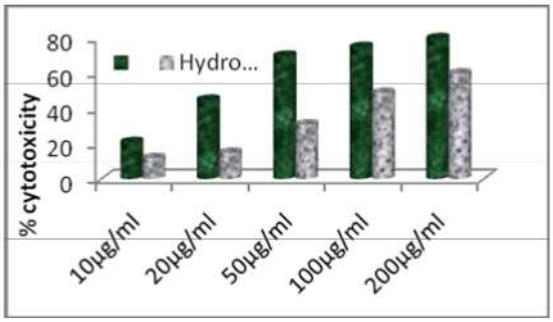


Figure 2: Cytotoxic activity of Chloroform and hydro-alcoholic extracts of *Bambusa bambos* in Erlich's Ascites Carcinoma cell lines

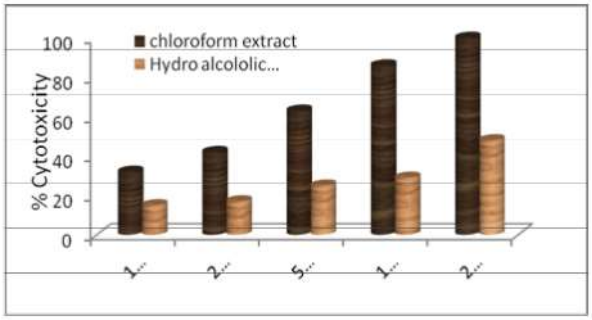


Figure 3: Cytotoxic activity of Chloroform and hydro-alcoholic extracts of *Bambusa bambos* in Dalton's Lymphoma Ascites cell lines.

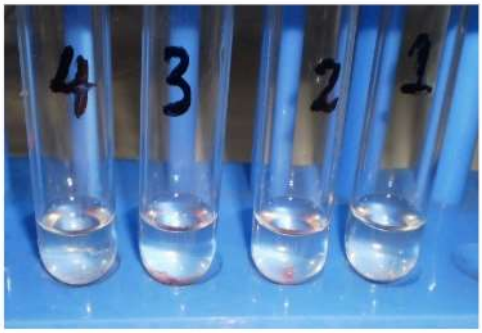


Figure 4: Effect of Chloroform extract on normal RBC – No Hemolysis

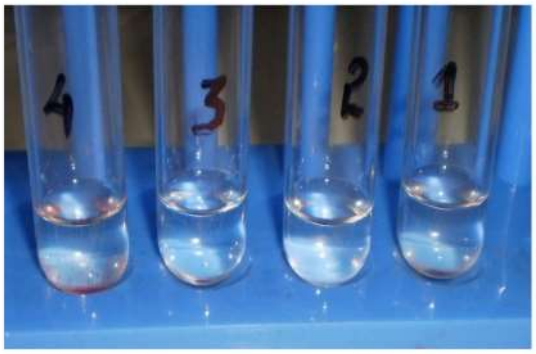


Figure.5: Effect of Hydro-alcohol extract on normal RBC – No Hemolysis

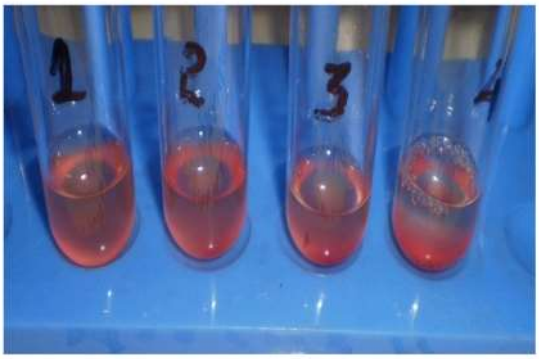


Figure 6: Effect of Mercuric chloride on normal RBC – Hemolysis

Cytotoxic activity

The results obtained from cytotoxicity study revealed that chloroform extract and hydro-alcohol extract showed remarkable (dose dependent cytotoxicity) anti cancer activity against both the test cell lines. Chloroform extract showed 80% cytotoxicity compared to hydro-alcohol extract which showed 60% cytotoxicity at the highest concentration of 200 µg/ml. In Dalton's Lymphoma Ascites (DLA) cell lines, chloroform extract showed 100% cytotoxicity compared to hydro-alcohol extract which showed 48% cytotoxicity at the highest concentration of 200 µg/ml.

Hemolytic activity

The hemolytic activity was performed to observe and confirm the safety of chloroform and hydro-alcohol extracts on to the normal RBC cell membrane. The results showed that there was no signs of hemolysis with test extracts when compared to the standard Mercuric chloride which showed hemolysis, indicated by red coloured solution because of the release of hemoglobin into extracellular solution, thus indicating the safety of test extracts on normal cell membrane.

DISCUSSION

The data presented clearly indicates that *Bambusa bambos* possess no significant hemolysis which provides the evidence that the extracts do not possess any toxicity to normal erythrocyte cells. The plant also possesses potent anticancer activity. *Bambusa bambos* may be useful as an anticancer agent and thus help in the treatment of many diseases mediated by reactive oxygen species. Plant derived natural products such as flavonoids, terpenes, alkaloids, polyphenols etc. have received considerable attention in recent years due to their diverse pharmacological properties including anti-oxidant, cytotoxic and cancer chemo-preventive effects[6]. The plant is known to contain flavonoids and alkaloids and phenolic acids as its chemical constituents [11]. Literature search on the published matter on *Bambusa bambos* revealed the presence of digestible crude proteins like lysine, methionine and Betaine in the aerial parts of the plant. Six phenolic acids viz., Chlorogenic acid, Ferulic acid, Coumeric acid, Protocatechuic acid, Vanillic acid and Coffeic acid were also identified in the aqueous extract of *Bambusa bambos* matured leaves [12] (Kundu, 2011). Phenolic compounds are considered to be the most important antioxidants of plant materials. They constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators. Antioxidant activity of phenolic compound is based on their ability to donate hydrogen atoms to free radicals. In addition, they possess ideal structural properties for free radical scavenging properties [13](Sulaiman, 2011). Hence the potent anticancer activity of *Bambusa bambos* is probably due to the presence of these phenolic acids which are well known for their anti oxidative properties and would play a significant role in preventing and treating cancer.

CONCLUSION

The present invitro study highlights the anti cancer potential of *Bambusa bambos*. An attempt is made to correlate the activity with the Phytoconstituents of *Bambusa bambos*. However further in vitro and in vivo studies are required on isolated Phytoconstituents to explore the mechanism of action and to validate the traditional uses of Bambusa bambos.

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